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## THE ARTERIAL PRESSURE IN THE EYE.

By W STEWART DUKE-ELDER (*Plimmer Research Fellow*)

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University College, London*)

ALTHOUGH several observers have claimed to have measured the arterial pressure in the eye, no one has yet succeeded in doing so. With one exception all the methods of technique hitherto adopted depend on raising the intra-ocular pressure and simultaneously noting the behaviour of the arteries. The eye thus acts as a sphygmomanometer, marked pulsation of the arteries is taken as the diastolic pressure, and their complete obliteration as the systolic. Since, however, the column of blood in the central artery of the retina and in the ciliary arteries is immobilised by this procedure, the pressure obtained is not that of the intra-ocular arteries but the lateral pressure at the most proximal branching of the feeding vessel—the ophthalmic artery.

The intra-ocular pressure has been raised by two methods

1. By inserting a manometer into the eye, and raising the pressure therein. The points of maximum pulsation and cessation of pulsation have been estimated either as observed ophthalmoscopically in the retinal artery (Schoeler (1) mean pr.—70 mm. Hg, v. Schulten, (2) Diastolic, Systolic pr.—90–120, 100–130 mm. Hg rabbit) or by measuring the amplitude of the pulsations communicated by the arteries to the mercury column in the manometer (Weiss (3), D/S pr.—50–70, 80–110 mm. Hg, Wessely (4) D pr.—70 mm. Hg rabbit)<sup>1</sup>

2. By raising the tension of the intact globe by the application of external pressure, either by applying to the eye an air filled pressure chamber connected with a manometer and provided with a transparent window through which the retinal circulation can be observed (Bleidung (5), D/S pr.—64–75, 96–117 mm. Hg in man) or by a piston working against a standardised spring ("dynamometer"), the ocular tension at the time of appearance and disappearance of the retinal pulse being estimated by a tonometer (Bailliant (6)). This latter procedure has been used extensively clinically, and the results of different observers vary by over 100 p.c., being much lower on the whole than those derived from the former method, Bailliant gives—D/S pr.—25–30, 50–70 mm. Hg in man. Apart from the limited value of the tonometric method of recording pressures in

<sup>1</sup> Lullies and Gulkowitsch (*Schriften d. Königsberger gelehrten Ges.* Bd. 2 H. 2, 1924) appear to have followed a similar procedure. I have been unable to gain access to the original paper but from an abstract their results would seem to be D/S pr.—34–70, 92–105 mm. Hg rabbit



any other than a comparative sense, the eye, rendered tense by this procedure, is pressed back into the orbit, thus kinking and compressing the vessels behind it. Thus doubtless accounts for the lowness of the results obtained. In laboratory animals Baillart's results are higher ( $D/S$  pr—40/100 mm Hg cat). The discrepancy is probably explained, not on any normal pressure difference in the retinal arteries of the two, as Baillart claims, but by the fact that these animals have two ophthalmic arteries, one derived from the external and one from the internal carotid, which anastomose so freely that on the obliteration of one or other of them the intra-ocular circulation can be maintained. Whatever doubtful clinical value these results may be construed to have, considered merely as figures with a comparative and no absolute significance, they are no record of the retinal arterial pressure, and are valueless even as an index of the pressure in the ophthalmic artery or of the relation between the intra ocular pressure and the vascular pressures in the eye.

The pressure in the anterior ciliary arteries in man, as they are seen under the conjunctiva just before they enter the eye, has been measured by Seidel (7) and Hiroishi (8) by applying a pressure chamber connected with a manometer over them.  $D/S$  pr appeared as 30–45/55–75 mm Hg. Seidel assumed that the pressure of the arteries in the eye, particularly those in the ciliary body which govern the formation of aqueous, was necessarily less than this. But these vessels at their entrance into the eye are very minute and have undergone several sub branchings since leaving the ophthalmic artery, their pressure will therefore be presumably less than that of the central artery of the retina and the posterior ciliary arteries which enter the eye directly from this parent vessel. Moreover there is a considerable amount of physiological evidence that the small anterior vessels play a subsidiary part in the maintenance of the ocular circulation. In some animals they are absent, and when they are present no markedly deleterious effects result from their obliteration, while section of the posterior ciliaries, two of which run without subdivision up to the region of the ciliary body, is followed by complete hypotony and widespread degenerative changes even involving structures as anteriorly situated as the cornea.

1 *The arterial pressure in the eye* The arterial pressure in the branches of the retinal artery was measured by a micro-injection method carried out by the introduction into their lumen of a micro-pipette of the type described by Barber (9), its movements being controlled by a micro-manipulator<sup>1</sup> modified from that elaborated by Chambers (10). The animals employed were cats. It was seen in a previous paper (11) that for the measurement of the venous pressure of the eye, dogs were the most suitable, but in these animals the retinal vessels are ensheathed and partially obscured by neuroglia, only fine branches being visible on the optic disc. The retina of the cat most nearly resembles that of man, and in it (usually three) comparatively large arteries, each flanked by a vein, are readily seen.

Anæsthesia was induced by ether, and maintained by intravenous chloralose. The lower lid was reflected along with the soft tissues, the periosteum elevated, a V-shaped piece of bone removed from the lower

<sup>1</sup> This was made at the suggestion of Prof Leonard Hill by Dr Schuster at the National Institute for Medical Research.

orbital margin, and the under-surface of the globe of the eye exposed. The animal's head was then securely clamped (A, Fig 1) in a suitable

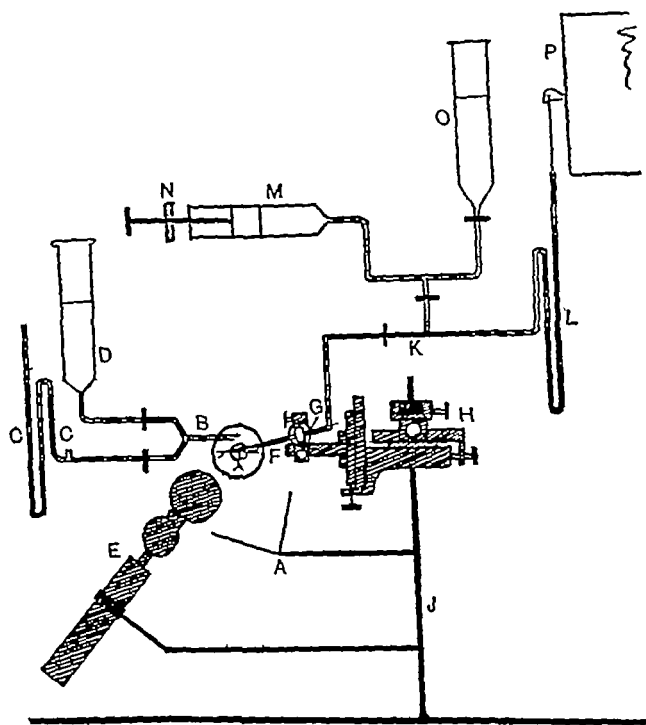


Fig 1 Pressure in intra ocular arteries

position. A hollow needle (B) of large bore to prevent any valvular action, and provided with a side-arm, was inserted into the vitreous, and connected to a compensated mercury manometer (C) and saline reservoir (C') by means of a capillary tube containing an air bubble to serve as an index of equilibrium. The side-arm of the needle was connected with a second reservoir filled with physiological saline (D). The normal intra-ocular pressure was taken in the usual way by means of the compensated manometer, the reservoir (D) was then raised to a corresponding height and its connection with the eye opened—by this means the intra-ocular pressure was kept at its normal level throughout all the subsequent experimental manipulations. A self-illuminating ophthalmoscope (E) was then adjusted to obtain a good view of the fundus by the direct method, and was clamped into position. The sclerotic was then pierced well behind the equator of the eye by a fine

metal needle, and the micro-pipette (*F*), carrying a collar (*G*), was inserted immediately on the withdrawal of the needle into the back of the globe through the hole thus made. The pipette was made by drawing out a glass capillary tube over a micro-burner into a needle with a rapidly tapering tip, and converting this into a pipette by jamming its point against a cover-glass until it broke off with a tip of such a size as would enter the branches of the retinal artery (about 0.1 mm diam at the disc), and when it was in place would allow the circulation therein to proceed unimpeded. Under the guidance of the ophthalmoscope, the tip was made to approximate closely to the branch of the artery on the side of the disc opposite to that at which the pipette was inserted, and when in this position, the micro-manipulator (*H*) was brought into position, and the collar securely fixed by a screw. The manipulator, the animal's head, and the ophthalmoscope were all clamped to the same support (*J*) in order to secure rigidity of adjustment. The manipulator was provided with a system of adjusting devices, governed by screws opposed by springs, so that the movements of the tip of the pipette could be accurately and continuously controlled in any direction, and maintained securely in any desired position. Under observation through the ophthalmoscope the tip was thus made to enter the lumen of the artery by adjusting the screws on the manipulator. The micro-pipette was connected by tubing (*K*) to a mercury manometer (*L*), the whole system being filled with a solution of methylene blue in physiological saline. Any desired pressure was made to act upon the pipette tip by means of a syringe (*M*) whose movements were accurately controlled by a milled screw adjustment (*N*) on the piston, while a constant supply of solution was maintained by recharging the syringe from a reservoir (*O*) and suitably adjusting the stop-cocks.

While the pipette was being manipulated into the artery, the system was kept at the normal intra-ocular pressure so that none of the methylene blue escaped into the eye and obscured the field. Once it was introduced, blood was seen to flow up into it. The pressure was then raised until the methylene blue solution flowed continuously into the artery—this was easily seen ophthalmoscopically. The pressure was then lowered until a slight stoppage of this flow occurred at systole, when a little blood tended to enter the tip of the pipette. On lowering the pressure further the dye and the blood fluctuated in the tube, and no flow took place, until a point was reached when an almost steady flow of blood into the pipette occurred with a periodic stoppage at diastole, when a small spurt of the dye entered the vessel. These

two pressures were recorded by marking their height on the kymograph (*P*), a correction factor being added to allow for the influence of a column of saline equal to the differences in level between the manometer and the eye

Several series of readings were taken in each experiment, the mean of which are given in Table I

TABLE I

No of cat	Intra ocular pressure mm. Hg	Pressure retinal artenes mm. Hg			
		Systolic	Diastolic	Mean pressure interpreted as	
				S - D	
				$\frac{S - D}{2}$	
1	22	91	65	78	
2	25	88	59	73 5	
3	29	94	69	81 5	
4	20	86	65	75 5	
5	23	83	63	73	
Average		24	88 5	64	76

2 *The pressure in the ophthalmic artery* The pressure in the ophthalmic artery was measured by a method modified from that adopted by previous workers by making use of the eye as a natural sphygmomanometer. In the criticism of their results it was seen that any interference with the circulation behind the globe by pressure applied to the intact eye entirely vitiated the results, and therefore a manometric method of raising the pressure was adopted. The pulse was both observed by the ophthalmoscope and recorded by the oscillatory method. The pressure pulse of the arteries is communicated by the incompressible contents of the eye to the elastic sclerotic as a volume pulse. This pulse is due to the intra-ocular arteries, and is not communicated from the orbital vessels, since, on inducing local endocular hyperæmia (by subconjunctival saline injections, etc., Wessely(4)) the ocular pulse increases proportionately, while a simultaneous carotid tracing shows no change. Inasmuch as the chorioidal vessels form 8/10 of the entire circulation of the eye, the pulse will be largely due to their influence, which is demonstrated by the fact that clinically, in cases of embolism of the retinal artery, when this vessel is occluded, the pulsations of the eyeball, as shown by the lever of a tonometer, proceed as usual. The pressure in the ophthalmic artery is thus measured through the central artery of the retina by the ophthalmoscopic method, through the posterior ciliary arteries by the oscillatory one.

In the first method it was found to be very difficult to observe the retinal circulation sufficiently exactly to get accurate end-points

V Schulten inserted the manometer into the vitreous, but here the difficulty of blocking the point of the needle by valve action complicates accurate readings when the pressure is made to vary. When the needle is immersed in the aqueous, the corneal astigmatism brought about by its insertion tends to blur the view of the fundus, an effect which is increased by the raised pressure tending to displace and alter the dioptric properties of the lens. The end-points, moreover, are not sharp. In the previous series of experiments it was easy to differentiate between the methylene blue and the red blood, but here, while arterial pulsation was seen to increase until pressures of 70 to 90 mm Hg were reached, it was very difficult to say with certainty where the maximum occurred. Similarly, at 110 to 120 mm Hg the blood flow in the vessels was seen to stop, and at pressures 5 to 10 mm Hg above this the vessels usually to a greater or less degree became collapsed and flattened out, but here again the end-point is indefinite, and does not lend itself to objective exactitude.

Reliance was therefore placed more upon the oscillatory method. Since the pulsation is communicated to the sclerotic, its magnitude has been estimated by the excursion of the lever of a tonometer placed upon the globe. But the distensibility of the coats of the eye (Koster<sup>(12)</sup>) decreases as its tension is raised, and the pulse is correspondingly damped, any method, therefore, which purports to compare the variations of the ocular pulse with increasing pressure by measuring its amplitude as communicated to the sclerotic is progressively more inaccurate as the tension rises. This error is overcome by opening the eye and putting its fluid contents in free communication with a rigid fluid system where the oscillations can be studied, conditions being so arranged that they are magnified to the greatest degree possible.

A manometer needle (*A*, Fig 2) of large bore (1 mm) to ensure free communication of the oscillations, and provided with a side-arm, was inserted through the cornea, the needle point being kept in the periphery of the anterior chamber to allow ophthalmoscopic examination of the fundus. From the side-arm a tube (*B*) was connected up with a compensated mercury manometer (*C*) with a reservoir (*C'*). The tube was connected with a syringe (*D*) whose piston was controlled by a screw adjustment (*E*), and the whole filled with saline, a constant supply of which was obtained from a reservoir (*F*). The straight end of the manometer led directly into a fine capillary tube (*G*) lying horizontally, on to which was attached a scale graduated arbitrarily. The capillary was connected by rubber tubing to a glass tube (*H*) containing saline, which

acted as a reservoir and could be raised or lowered by a pulley. This reservoir was made as small as was convenient in order to reduce the

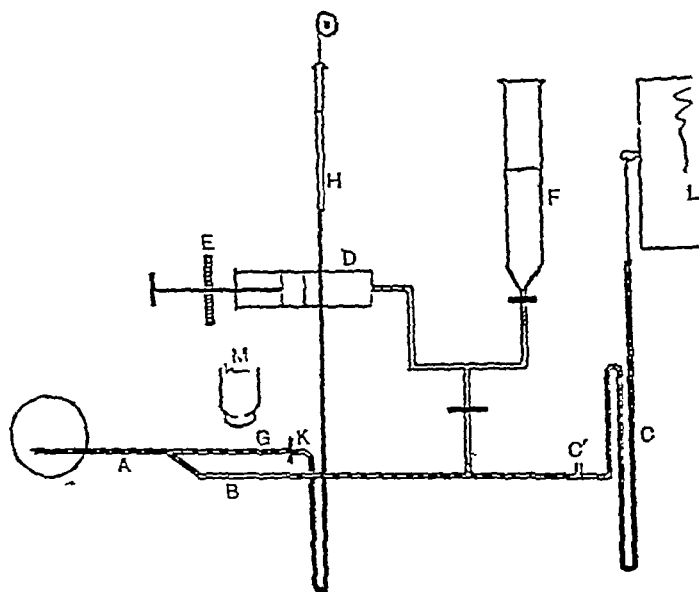


Fig. 2. Pressure in ophthalmic artery

inertia of the fluid column in the capillary (G), and make the oscillations as free as possible

When the manometer was inserted, the reservoir (H) was shut off by a stop-cock (K), an air bubble was then introduced into the tube (B), and the pressure in the system adjusted by manipulating C' until the bubble was stationary, the normal intra-ocular pressure being thus recorded on the manometer. Communication with the reservoir (C') was then closed and that with the reservoir (H) opened after its height had been adjusted to correspond with that registered by the manometer. The pressure in the syringe was then slightly increased while the level of the reservoir (H) was kept unaltered so that the air bubble travelled slowly down the tube (B) and along the capillary (G). Having arrived here it was kept constantly in the same place by raising or lowering the reservoir coincidently with any subsequent manipulations of the syringe. When the pressure in the eye was varied by means of the syringe the behaviour of the retinal artery was observed ophthalmoscopically and the excursion of the air bubble in the capillary was noted through a microscope (M). The points of maximum oscillation

and cessation of oscillation were marked on the kymograph (*L*), and were subsequently measured, corrections being applied to compensate for a column of saline equal to the difference in level between the eye and the manometer

There follows the protocol of a typical experiment, the figures expressing the amount of oscillation refer to the divisions of the scale and are therefore arbitrary and of purely comparative significance

TABLE II

Cat Intra ocular pressure mm Hg	Ophthalmoscopic appearances	Amplitude of oscillation
26	Vessels normal, no pulsation	4-5
28	Venous pulsation at disc arteries normal	4-5
40	Veins definitely engorged, arteries pulsating slightly	6-7
60	Veins begin to become constricted, arteries pulsating	8-10
70	Arterial pulsation increasing	11-12
75	" " "	14-15
78	Arteries pulsating maximally(?)	18-20
80	Arterial pulsation marked	16-17
90	" " less	9-12
110	Veins constricted, arteries filling at systole	6-7
120	Veins collapsed, arterial pulsation ceased	0
125	Arteries barely visible	0
Mean carotid pressure 114 mm Hg		

Taking the point of maximal pulsation as the diastolic and that of cessation of pulsation as the systolic pressure, the results of a series of six experiments are given in Table III

TABLE III.

No of cat	Intra ocular pressure mm Hg	Pressure in ophthalmic artery mm Hg			Mean carotid pressure mm Hg
		Diastolic	Systolic	Mean pressure interpreted as	
				$\frac{S + D}{2}$	
1	26	78	120	99.5	108
2	29	85	129	107	118
3	26	74	106	90	96
4	23	81	110	95.5	100
5	25	73	116	94.5	103
6	20	80	109	94.5	104
Average	25	78.5	115	97	106

3 *The mean pressure as measured in the carotid artery was registered by a mercury manometer. Only in one case (Table IV) were the four measurements taken in the same animal, as a general rule the length of time occupied by the manipulations and the consequent change in the animal's condition rendered comparative values of little account. If it be permitted to transpose the venous pressures in the eye of the dog*

as determined in a previous paper(11) we get the following pressure relationships

TABLE IV

		mm. Hg		
		Systolic	Diastolic	Mean
Ca <sup>+</sup>	Pressure as measured in carotid artery (right side)	—	—	105.5
	Pressure in ophthalmic artery (right)	119	80	99.5
	Pressure in retinal artery (left) i.e. in first arterial branching in eye	86	65	75.5
Dog	Pressure in venous exits	—	—	21.5
Ca <sup>+</sup> and dog	Intra-ocular pressure	—	—	20
Dog	Pressure in extra-ocular veins	—	—	12.2

It is seen that the pressure in the ophthalmic artery is only a few mm. Hg below that measured in the carotid<sup>1</sup> that is, below the aortic pressure, and that in the first branching inside the eye a larger fall of about 25 mm. Hg or almost 25 p.c. of the total pressure occurs. Between this and the veins a large fall of 54 mm. Hg takes place. This distribution of the pressure gradient is in conformity with that encountered in other parts of the body, where the arterial pressure is maintained at a fairly constant high level until the smaller arteries are reached.

The systolic pressure in the carotid was determined with the same technique as that employed in measuring the pressure in the retinal arteries by the insertion of a pipette. A mean pressure of 108 mm. Hg was found to correspond with a systolic maximum of 150 mm. The large amplitude of the pulse pressure and the impossibility of seeing the methylene blue solution entering this artery however, rendered the method unsatisfactory for obtaining correct readings of the diastolic pressure. To obtain a satisfactory diastolic end-point the arterial wall must be so thin as to be transparent. For the same mean pressure it is probably about 70 mm. Hg.

### DISCUSSION

From the physiological standpoint the main interest in these vascular pressures is their relation to the formation of the aqueous humour. This is associated mainly with the vessels in the ciliary body and the iris. From a theoretical point of view it would have been preferable to have obtained measurements of the arterial pressures here. In the cat the circulus arteriosus iridis major, which supplies the ciliary processes and iris directly, instead of lying inaccessible at the base of the iris as in man, lies in the iris itself and with sufficient magnification is readily seen particularly on the nasal side. To introduce a pipette into this

<sup>1</sup> The pressure in the carotid artery was taken last there is probably, therefore, an error comparatively, due to a decline in the animal's condition.



vessel, however, was found to be very difficult or impossible on account of the mobility of the supporting structures, while the immediate and high rise in the intra-ocular tension which followed penetration of the iris—due presumably to vaso-motor reflexes from this highly innervated structure—seemed to render any such attempt useless. This vessel, however, is directly formed from the long posterior ciliary arteries. Since, other things being equal, arterial pressure decreases in proportion with the number of branchings and the size of the lumen of the vessels, and since the long posterior ciliary arteries and the central artery of the retina are both direct branches of the ophthalmic and are of the same order of size, it would seem probable that the pressure in this arterial circle would approximate that in the branches of the central artery of the retina. Moreover, in a previous paper (11), the close relationship between the pressures in the two circulations—uveal and retinal—under physiological variations has already been pointed out. It is probable, therefore, that we can assume with a fair degree of certainty that the pressures measured in the branches of the retinal artery are not far removed from those in the ciliary body.

We may thus assume that in the ciliary body the arterial mean pressure is about 75 mm Hg, that the venous pressure is about 25 mm Hg, while the intra-ocular pressure is about 24 mm. There is thus a fall in the vascular system of about 50 mm Hg.

Although the blood-pressure in the corresponding organs of the higher animals are generally taken as proportionately equal, the objection may be raised in transferring these measurements to the case of man that the arterial supply of the eye is different in the two cases. It has been noted that in the usual laboratory animals the ophthalmic arteries are derived from both the internal carotid and the external or from an anastomic branch between them. In man it is derived only from the internal. It is very probable, however, that the difference, if any, is in the direction of a higher pressure in the ophthalmic artery of man. In him this vessel comes off as a direct terminal branch of the internal carotid, virtually from the circle of Willis, a vessel which the whole vaso-motor mechanism of the body strives to keep at a high pressure at all times. Further, immediately after the ophthalmic artery has left the internal carotid, the latter vessel constricts, the narrowing being out of proportion to the diminution of its blood stream as judged by measurements of the cross-sections of the vessels (Whitnall (13)), a provision which, by reducing the calibre of the main vessel distal to this important branch and thus damming up the blood stream, will

favour the passage of blood down the latter and maintain a high pressure in it. It is probable, therefore, that in man the pressure gradient from arteries to veins is more than 50 mm Hg.

No method has yet been devised to measure the capillary pressure in the eye. To do so would seem almost impossible, for we have previously seen<sup>(11)</sup> that any intra-ocular manipulation or pressure applied to the eye at once affects the venous pressure and with it the capillary pressure, the three tending to rise coincidentally. This consideration at once rules out the estimations of Niesnamoff<sup>(14)</sup> and Dieter<sup>(15)</sup>. In default of a direct measurement we must rest content with an indirect estimation based on the measurements of the pressures in the arteries and veins—if, indeed, with their continually changing conditions and wide range of variation it is reasonable to speak of a capillary pressure at all. Most recent estimations show that the capillary pressure is very low, but the majority of these have been undertaken in the skin most of whose capillaries appear to be venous in nature (Krogh<sup>(16)</sup>). There seems little justification for applying them to the general circulation, and none for applying them to the specialised conditions of the eye. On the other hand it would appear from the work of Dale and Richards<sup>(17)</sup> and Burn and Dale<sup>(18)</sup> that the peripheral resistance is not limited to the arterioles, and that the assumption that an abrupt fall in pressure takes place in this part of the circulation is unwarranted, but rather that the fall in pressure is evenly distributed between the smallest arterioles and the first capillaries without a sharp line of demarcation between them. That such a conception is probable is suggested by the influence of the mechanical action of the corpuscles and the contractility of the capillaries as demonstrated by Krogh<sup>(16)</sup>, Lewis<sup>(19)</sup> and others, and it seems to be substantiated by the recent work of Landis<sup>(20)</sup>, who, using a micro-injection technique such as is followed in this paper, has shown that in the frog's mesentery the fall of pressure does not cease abruptly at the arterioles, but continues to the venous capillaries before flattening.

When it is remembered that the intra-ocular pressure is 20–25 mm. Hg, it would seem that the arterial and venous pressures in the eye bear a relation to the chamber pressure similar to that which the vascular pressures do to the tissue pressure throughout the body generally. The tissue lymph contains about one-half the quantity of colloids that are found in the blood, while the aqueous is practically protein-free. Consequently, if the fluids of the eye can be formed without the intervention of a "secretory" mechanism, the capillary pressure in the eye must

exceed the intra-ocular pressure by more than the difference that obtains generally between capillary and tissue pressure. In round figures a difference of 30 mm Hg must exist in the eye instead of 15 mm elsewhere. There is every indication that this may be so. In the first place, the ciliary arteries seem to be anatomically peculiar in that they break up almost at once into a rich net-work of capillaries (Fusita<sup>(21)</sup>), which appear to be capable of such extreme distension of their lumen as to allow the passage of ten corpuscles at a time. In these the lateral pressure will be capable of rising to a considerable height. Again the veins are physiologically constricted at their exits from the eye, and the whole system is confined under a considerable tension within a feebly distensible case, the sclerotic, which will make the vessels approximate in their behaviour to a system of rigid tubes. These considerations will all tend to throw the site of the fall of pressure further towards the veins, and make it probable that the pressure in the arterial capillaries rises at least 30 mm Hg higher than that in the chamber of the eye, i.e. to a total of about 50 to 55 mm Hg. The vascular pressures, therefore, although by themselves they prove nothing, make it possible that the aqueous humour is formed purely by a process of dialysation.

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# THE EFFECT OF CO<sub>2</sub> ON THE ABSORPTION OF ALCOHOL AND THE INFLUENCE OF ALCOHOL ON THE DIFFUSION OF CO<sub>2</sub> IN THE SMALL INTESTINE

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IN a former investigation<sup>(1)</sup> it was shown that CO<sub>2</sub> increased the absorption of alcohol by the gastric mucous membrane in the cat and that the presence of alcohol in the stomach had the curious effect of apparently making the mucous membrane of the stomach permeable in one direction only. The present investigation was undertaken to determine whether these effects were also exhibited by the mucous membrane of the intestine. At the same time the opportunity was presented of making a comparison between the effect of alcohol on stomach and intestine.

*Method* The method employed was similar to that in dealing with the stomach. The animal (cat) was anaesthetised with ether and decerebrated or pithed by Langley's starch injection method, artificial respiration being generally applied. Eight inches from the pylorus a piece of gut 55 cm long was measured and clamped off. At each end a cannula connected with a tap was ligatured into the loop, and the loop was washed out with warm unbuffered Ringer's solution. The upper cannula was connected with a side tube between it and the tap, and through this tube the liquid to be investigated was introduced and kept at a constant pressure of 5 cm. of water.

The basic experiments as in the case of the stomach were done with unbuffered Ringer only. The table given below shows results which enable us to compare the absorption of Ringer only from the stomach

TABLE I

	Intestine			Stomach		
	Ringer introduced c.c.	Liquid removed c.c.	Absorption P.c.	Ringer introduced c.c.	Liquid removed c.c.	Absorption P.c.
12	7.8	2.3	70	—	—	—
13	22.6	Trace	100	54.5	56	-1.5
14	15.8	7.4	53	51.5	51.5	0
15	24	18	25	68.2	66	-2.2
16	36.3	7	80	53	53	0

and intestine in the same animal. The intestinal absorption though very variable is always considerable. These experiments are similar in results to those which have been obtained by former observers, the reason we introduce them is that the animals having been either decerebrated or pithed the influence of the anæsthetic is largely eliminated.

*Absorption of alcohol in the intestine* The alcohol experiments were done on the same lines as in the case of the stomach only, i.e. each experiment consisted of two parts, each part was only of one  $\frac{1}{2}$  hour's duration. In (a) about 8 p.c. alcohol in unbuffered Ringer was used and in (c) the same alcoholic solution to which about 44 p.c. of  $\text{CO}_2$  had been added. In some experiments solution (a) was used first and then solution (c) and in others the solutions were used in the reverse order.

TABLE II.

Exp		C c alcohol absorbed in intestine	P c liquid absorbed	P c $\text{CO}_2$ at beginning	P c $\text{CO}_2$ at end of experiment
1	(a)	1.565	70	0	?
	(c)	2.38	24	—	?
2	(c)	1.53	70	About 44	?
	(a)	1.08	10	0	?
3	(c)	1.287	31	40	17
	(a)	1.121	6	0	16
4	(a)	1.0	16	0	20
	(c)	1.0	0	43	20
5	(c)	2.03	62	43	39
	(a)	1.399	12	0	35
6	(a)	0.755	Slight secretion	0	14
	(c)	0.686	"	43	17
7	(c)	1.275	16.4	44	14
	(a)	0.696	Slight secretion	0	16
8	(a)	1.04	10	0	12
	(c)	1.11	0	48	12
9	(c)	1.06	35	0	32
	(a)	1.06	0	43	20
10	(a)	0.714	40	0	16
	(c)	0.513	0	48	20
11*	(x)	—	11.4	0	14
	(y)	—	9.3	24	19
12*	(y)	—	7.2	56	31
	(x)	—	7.9	0	32

\* In these experiments no alcohol was used, otherwise they were similar.

From the above table, which presents a sample of the results obtained from numerous experiments, it is clear that as far as  $\text{CO}_2$  is concerned either the mucous membrane of the small intestine is acting merely as a passive membrane, or the  $\text{CO}_2$  is the result of secretion. We

endeavoured to settle this point by estimating the carbonate ion content of the boiled out liquid, assuming that, if the  $\text{CO}_2$  present at the end was some of the gaseous  $\text{CO}_2$  introduced, then the liquid on boiling out would have been free of  $\text{CO}_2$ , whereas, on the other hand, if there was a taking up of baryta after boiling then the  $\text{CO}_2$  must have been fixed either as  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$ . It was difficult to estimate the  $\text{CO}_2$  after boiling because of the very mucilaginous character of the liquid, but the results, though too unreliable to emphasise figures, suggest that the  $\text{CO}_2$  was fixed, probably as  $\text{NaHCO}_3$ . This fact was deduced because the alkalinity of the liquid increased on boiling though the  $\text{CO}_2$  decreased. The explanation is, we consider, that a little  $\text{Na}_2\text{CO}_3$  was secreted by the intestinal mucosa and this then met a large excess of  $\text{H}_2\text{CO}_3$  in the lumen,  $\text{NaHCO}_3$  was formed, which on boiling lost  $\text{CO}_2$  and the liquid therefore became more alkaline.

With regard to the absorption of alcohol in the presence of  $\text{CO}_2$  there seems to be a tendency for more alcohol to be absorbed in the presence of  $\text{CO}_2$ , though the effect is not nearly so marked as in the stomach.

In the second part of the experiment peristalsis always occurs and usually less alcohol is absorbed, but the difference is less marked when there is  $\text{CO}_2$  in the liquid in the last part of the experiment than when it is introduced first.

Restating some of the results already presented in Tables I and II it can be seen that the presence of alcohol has an effect on the actual absorption of liquid.

*Absorption of liquid from the intestine*

P c absorption in 1 hour		P c absorption in successive $\frac{1}{2}$ hours			
Ringer alone	Ringer + alcohol	Ringer alone		Ringer + alcohol	
		1st $\frac{1}{2}$ hour	2nd $\frac{1}{2}$ hour	1st $\frac{1}{2}$ hour	2nd $\frac{1}{2}$ hour
70	25	—	—	—	—
100	16	38	30	25	0
50	31	75	68	20	5
25	20	22	22	27	0
80	37	—	—	—	—

We can conclude that alcohol does not hasten the absorption of liquid, on the contrary, the figures for the absorption of liquid containing alcohol are usually considerably lower than in the case of unbuffered Ringer alone. Moreover, alcoholic absorption seems to slow down the absorption of liquid very rapidly, since in the second part of an experiment with alcohol there is very often no absorption at all, whereas in the second half of an experiment with Ringer alone absorption is nearly

as good as it was in the first half. It might be suggested that the volume of fluid in the intestine does not change in the second half of an experiment with alcohol because secretion balances absorption, but the small amount of carbonate present in the fluid removed indicates that this is not the explanation and that absorption is retarded.

From all these considerations we may conclude that the addition of alcohol to water diminishes its efficiency in quenching the thirst.

#### SUMMARY

1 The effect of  $\text{CO}_2$  is to increase the absorption of alcohol slightly, though not so markedly as in the stomach.

2 It cannot be stated whether the presence of alcohol affects the diffusion of  $\text{CO}_2$  because of the possible secretion of  $\text{Na}_2\text{CO}_3$  into the lumen.

3 Alcohol appears to retard the absorption of liquid in the intestine.

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# INSULIN AND THE PRODUCTION OF ACETONE BODIES BY THE PERFUSED LIVER

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ONE of the striking features of the action of insulin in severe diabetes is the way in which it brings about a cessation of the formation of the acetone bodies. This is usually ascribed to a return of the power of the organism to utilise carbohydrate to a greater extent than is possible without insulin and explanations have been advanced which suggest that at a certain stage in their metabolism, fats require the assistance of carbohydrate for their complete oxidation. There is, however, no direct evidence in favour of views of this kind.

The isolated dog's liver when perfused with defibrinated blood has been shown by Embden and others to produce small amounts of acetone bodies but much larger amounts if ammonium or sodium butyrate was added to the blood. The perfusion of the isolated liver with blood containing small amounts of sodium butyrate appeared to us therefore to offer some prospect of gaining an insight into the antiketogenic action of insulin.

Burns<sup>(1)</sup> has shown that when sodium acetoacetate is injected into the decapitate cat with kidneys removed, the rate of fall of the total acetone of the blood is independent of the injection of insulin. The possibility remains that insulin may affect the production of the acetone bodies from their precursors in the liver.

Further observations in connection with acetone formation after injection with insulin have been made by Collip<sup>(2)</sup> and by Robertson and Anderson<sup>(3)</sup>. The former showed that acetone bodies appeared in the urine of normal rabbits which had been rendered hypoglycæmic with insulin and came to the conclusion that in the condition of carbohydrate starvation induced by the removal of available glucose, the liver commenced to draw on the source of energy normal to it under these conditions, i.e. protein and fat, the latter giving rise to the urinary acetone. The latter authors endeavoured to correlate hypoglycæmic coma with acetoacetic acidosis, but could find no evidence in favour of this view.

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Recently v Fazekas(4) has shown that administration of insulin in cases of diabetic coma results in a diminution of  $\beta$ -oxybutyric acid, the acetone + acetoacetic acid, and the blood-sugar being for a considerable time unaffected

*Acetone production in cat's liver perfused with foreign blood* In the first series of experiments, cats' livers were perfused *in situ* with de fibrinated ox or pig's blood In order to eliminate the effect of prolonged ether narcosis, the animals were decerebrated before the commencement of the experiment The blood, warmed to  $38^{\circ}\text{C}$ , entered the portal vein from a reservoir 15 cm above the table The tributaries of the portal vein and the inferior vena cava below the diaphragm were ligatured, and the blood was drawn off by means of a cannula in the inferior vena cava above the diaphragm Arterialisation was effected by bubbling oxygen through the blood in the collecting vessel Usually no difficulty was experienced due to clots forming in the liver vessels, but it was observed that the mechanical obstruction to the flow of blood, evinced by the swelling of the liver and falling off of the rate of perfusion, gradually increased as the perfusion proceeded This effect was more noticeable in the case of ox than pig's blood The rate of perfusion was maintained, when possible, between 80 and 100 c c per minute The first blood sample was taken immediately a constant rate and temperature of perfusion were established, and the second after one hour's perfusion, the blood-sugar, acetone and acetoacetic acid (together), and  $\beta$ -oxybutyric acid being determined in each sample In this series, the acetone bodies were determined by Shaffer's method(5) on 50 c c samples, and the blood-sugar by Maclean's method Since in the majority of the experiments, butyric acid was added to the perfusion fluid, it was found necessary to replace Shaffer's method of aldehyde removal by Masuda's silver oxide method(6), because butyric acid yields considerable quantities of acetone when boiled with hydrogen peroxide in alkaline solution The liver fat was approximately estimated at the end of perfusion by saponification, acidification, extraction with light petroleum, and titration in alcohol with  $N/20$  sodium hydroxide The figures in the tables give the percentage of fat reckoned on the moist weight of the liver after perfusion

The acetone body formation per litre of blood without addition of sodium butyrate is given in Table I

In Tables I-III (a) = acetone + acetoacetic acid,

(b) =  $\beta$ -oxybutyric acid

These and the "total acetone bodies" are all calculated as mg acetone per litre of blood

TABLE I. Without added sodium butyrate

Exp	Weight of liver gram.	Fat content %	Sample 1		a-b	Sample 2		a-b	Total acetone bodies formed
			a	b		a	b		
1	53	14.0	28.2	59.6	57.8	48.5	97.0	145.5	57.7
2	100	4.4	11.1	50.7	61.8	23.3	64.4	87.7	25.9
3	50	5.5	8.1	31.5	39.6	19.7	31.5	51.2	11.6

The above figures may be compared with those obtained with dogs' livers by Friedmann(7), 19.4, 61.7, 63.5, 84.0, and by Dakin(8), 32.5 mg per litre. Taking into account the relative sizes of the livers used, it is seen that the "total acetone" production is greater in cats than in dogs.

When 0.2 p.c. of butyric acid (as sodium butyrate) was added to the perfusing blood, the acetone production was considerably greater as shown in Table II. In experiment (6) the animal was pregnant, and it was thought possible that the high result was due to the condition of fatty infiltration associated with pregnancy. The result obtained on another pregnant animal (7), gave a similar high value and it seems likely that the acetone production of the liver of the pregnant animal is greater than that of the normal.

TABLE II. Perfusion with added butyric acid.

Exp	Weight of liver gram.	Fat content %	Sample 1		a-b	Sample 2		a-b	Total acetone bodies formed
			a	b		a	b		
4	64	2.4	8.3	44.6	52.9	41.8	155.9	197.7	144.8
5	102	12.2	6.4	66.8	73.2	30.9	212.0	242.0	169.7
6	90	27.0	15.5	70.5	86.0	135.6	241.0	376.6	290.6
7	90	9.7	10.4	45.3	55.7	63.5	246.0	309.5	253.8

*The effect of addition of insulin to the perfusion fluid.* So far as could be determined from the results of three experiments, addition of insulin to the perfusing blood brought about no diminution in the acetone production. In experiment (8) 10 units of insulin were added at the commencement, in experiments (9) and (10), 30 units, and in addition, in experiment (10), the animal received 6 units of insulin before the experiment, and was anaesthetised when the hypoglycaemic symptoms were well established.

TABLE III. Perfusions with added butyric acid and insulin.

Exp	Weight of liver gram.	Fat content %	Sample 1		a-b	Sample 2		a-b	Total acetone bodies formed
			a	b		a	b		
8	78	3.5	12.8	22.2	35.0	81.4	163.3	244.7	209.7
9	62	3.2	13.8	46.7	60.5	57.0	213.2	300.2	239.7
10	83	11.4	14.0	51.3	65.3	74.2	214.6	288.8	253.5

The increase in acetone is, if anything, greater than in those perfusion without added insulin, but not greater than the variation that might be expected, hence it may be concluded that under the conditions of these experiments, insulin has no direct action on the liver in causing decreased output of acetone bodies. In all these experiments the changes in blood-sugar concentration were followed and in none of those in which insulin was added was there any indication of a difference from the control experiments without insulin. The glycogenolysis, indicated by the gradual rise in blood-sugar, was as marked in the insulin as in the control experiments. It was expected that insulin would have inhibited glycogenolysis and these results seemed to render it doubtful whether insulin would produce its normal action in an organ perfused with defibrinated blood from another species. It was therefore thought necessary to investigate this point further and attempts were made to obtain as a result of the action of insulin, an increased disappearance of blood-sugar in the hind limbs of cats perfused with ox or pig's blood. It has been shown by Burn and Dale<sup>(9)</sup> that in the decapitated and eviscerated animal, insulin causes a marked increase in the rate of disappearance of glucose from the blood and it was expected that a similar effect would be obtainable in the perfused hind limb.

*Perfusion of the muscle of the hind limbs* The perfusion was carried out with the same apparatus, the only necessary modification being a considerable increase in the height of the reservoir above the table for the purpose of obtaining the requisite arterial pressure. The operation was performed under ether anaesthesia. Double ligatures were applied to the rectum and inferior mesenteric artery, which were divided between the ligatures and the viscera retracted. A cannula was inserted into the aorta immediately above the iliac bifurcation, and a second into the vena cava at about the same level. A strong mass ligature was applied to the trunk below the kidneys and the animal completely severed above the ligature. The neural canal was plugged with cotton wool, but more or less hæmorrhage usually occurred from this point, the blood being collected and added to that from the vena cava.

It was found to be almost impossible to obtain a satisfactory flow when ox blood was used but less difficulty was experienced with pig's blood, which was therefore adopted. The procedure finally used was to wash out the blood vessels with 500 c c warm Ringer Locke solution containing 0.3-0.4 p c glucose, then with 500 c c of pig's blood which was discarded, the perfusion then being continued with a further 500 c c of pig's blood containing 0.3-0.4 p c of glucose.

As in the eviscerated animal, the muscle of the hind limbs was found to remove glucose from the blood at a fairly constant rate. If, at the end of an hour, 20 units of insulin were added to the blood immediately before entering the aorta, contrary to expectation, no alteration in the rate of removal could be observed. Of 17 experiments carried out as described above, 7 without added insulin showed a fairly constant rate of disappearance of glucose during the two hours, with added insulin at the end of the first hour, 2 experiments showed a slightly increased removal, but 8 showed no effect whatsoever. In order to determine more accurately any slight effect of insulin, a further series of experiments was carried out in which the fall of blood-sugar during two consecutive half-hour periods was determined. After allowing 30 minutes for the preparation to arrive at a constant rate of utilisation, duplicate blood-sugar estimations were carried out on two samples taken at an interval of two minutes, thus allowing a minimum error in the determination of the blood-sugar and the taking of the samples. Four similar estimations were made 30 minutes later, *i.e.* at the end of Period I. Insulin was then added, and four estimations were made after a second period of 30 minutes. The results are given in Table IV. Three control experiments of this type in which no insulin was added showed very little difference between the fall in the two periods, and three experiments with the addition of 20 units of insulin at the end of the first period also showed no greater drop in the second period than in the first. This showed definitely that under these conditions insulin was not exerting its normal action in causing the muscles to remove sugar from the blood at an increased rate.

TABLE IV (1) Glucose utilisation in isolated perfused muscle

Exp		Blood sugar %					
		Period I			Period II		
		Sample 1	Sample 2	Fall	Sample 1	Sample 2	Fall
11	Control	0.280	0.266	0.014	0.266	0.250	0.016
12	do	0.352	0.317	0.035	0.317	0.274	0.043
13	do	0.366	0.326	0.040	0.326	0.289	0.037
14	Insulin present in Period II	0.318	0.278	0.040	0.278	0.235	0.043
15	do	0.337	0.294	0.043	0.294	0.263	0.031
16	do	0.232	0.185	0.047	0.185	0.140	0.045

In these experiments the only serious deviation from the conditions obtaining in the decapitated and eviscerated animal lies in the use of foreign blood. The liver perfusions having been carried out under the

same conditions, however, it was necessary to repeat both these and the muscle perfusions with cat's blood

In order to minimise the number of animals necessary, a perfusion apparatus<sup>1</sup> (Burn and Dale(22)), operated by a Dixon pump, was adopted in place of that previously used. This enabled the perfusion to be carried out with 150 c c of blood and two cats sufficed for each experiment

*Muscle perfusion with animal's own blood* Two determinations were made with pig's blood in this apparatus in order to check the results obtained in the previous series. It was found that, owing to the smaller amount of blood in the circulation, the rate of fall of the blood-sugar was so great that the glucose was exhausted before the end of the second period. At the end of the first hour, therefore, sufficient glucose was added to bring the level of the blood-sugar to the same value as at the beginning of the first period. Ten minutes were then allowed to elapse for attainment of equilibrium, and samples taken for a third half-hour. Addition of insulin at the commencement of this last period had again no effect on the rate of fall of the blood-sugar, thus confirming the previous results

In the experiments in which cat's blood was used, the cat was bled from the aorta, and the vessels washed through with about 80 c c of Ringer-Locke solution. About 160 c c of defibrinated blood containing saline was obtained in this way from two animals

It was found that, using the animal's own blood, insulin considerably increased the rate at which the muscles removed glucose from the per-

TABLE V (2) Glucose utilisation in isolated perfused muscle

Exp	Blood used	Blood sugar %					
		Period I			Period II		
		Sample 1	Sample 2	Fall	Sample 1	Sample 2	Fall
17	Pig Insulin present in Period II	0.255	0.187	0.068	0.207	0.140	0.067
18	do	0.300	0.235	0.065	0.290	0.222	0.068
19	Cat Control	0.362	0.288	0.074	0.404	0.328	0.076
20	do	0.403	0.335	0.068	0.458	0.384	0.074
21	Cat Insulin present in Period II	0.454	0.365	0.089	0.700	0.510	0.190
22	do	0.385	0.263	0.121	0.370	0.174	0.196
23	do	0.313	0.194	0.119	0.283	0.150	0.133

<sup>1</sup> We are indebted to Drs Burn and Dale for a description of this apparatus details of which have now been published.

fusing blood This has been confirmed by Best<sup>(10)</sup> In two experiments in which no insulin was added, the fall in each period was approximately the same, but in three experiments in which insulin was added at the commencement of the second period the fall was definitely greater, in two of them markedly so The results are given in Table V

These figures show conclusively that the failure of insulin to bring about its characteristic action in the previous series is due to the perfusion having been carried out with foreign blood Whether this is a peculiarity of pig's blood alone has not yet been determined, but it is remarkable that the foreign blood does not interfere with the removal of glucose by the muscles although it inhibits the increased removal which insulin produces

*Perfusion of liver with animal's own blood* Since in the apparatus used in these experiments it was necessary to remove the liver completely from the body, all tributary vessels to the portal vein above the level of the cannula were carefully ligatured, a cannula was inserted into the bile duct or it was ligatured in order to prevent admixture of the bile secreted during the perfusion with the blood draining from the vena cava Before the perfusion was commenced the blood vessels were washed out with Ringer-Locke solution In order to inhibit the glycogenolysis which commenced with cessation of the circulation through the liver, the saline solution was cooled in ice to  $4^{\circ}$ , and the liver thus kept at a low temperature until the artificial circulation could be established

Samples for acetone body and blood-sugar estimation were taken 15 minutes after commencement of perfusion, and at 30 minute intervals Owing to the small volume of blood in circulation, 10 c c samples only could be taken, hence it was necessary to use the van Slyke method of total acetone body estimation<sup>(11)</sup> In the earlier experiments lactic acid was also determined by Clausen's method<sup>(12)</sup>

The total acetone production was found to vary to so great an extent with the individual liver that any effect of insulin could be detected only by carrying out a prohibitively large number of experiments Thus in four experiments in which sodium butyrate was added to the blood to give a concentration of 0.2 p c, the total acetone formation in one hour was 118, 128, 166.5, 141.5 mgs respectively In five similar experiments with addition of insulin at the commencement, the total acetone formation was 75.5, 128, 76, 190, 153 mgs respectively, i.e. in two instances less than, in one instance greater than, and in two instances about equal to the production in the control experiments It was necessary, therefore, to attempt to obtain both control and experimental

figures from the same liver, and for this purpose several experiments were performed in which the acetone production during two successive half-hour periods was determined. Although the acetone production was not perfectly steady during the course of the perfusion, the amounts formed during the two periods were sufficiently nearly equal to make it possible to detect any considerable effect of insulin added at the commencement of the second period. The following results show that no effect, either in the sense of increase or decrease of acetone production, results from the addition of 20 units of insulin at the end of the first perfusion period.

TABLE VI. Acetone body production, cat's liver perfused with cat's blood containing sodium butyrate.

Exp		Fat %	Glycogen %	Total acetone produced	
				Period I mg	Period II mg
24	Control	3.2	0.05	50	46
25	do	14.5	1.0	52	68
26	do	8.0	0.1	51	39
27	Insulin present in Period II	2.5	0.1	72	80
28	do	9.3	0.5	66	72
29	do	2.5	0.1	41	36

The mean acetone production in the controls was 51 mg. in each period, in the experiments with insulin, 60 mg. in the first period, 63 mg. in the second period, showing conclusively that insulin does not affect acetone production in the perfused cat's liver.

*Perfusion of dogs' livers.* The technique was identical with that employed in the case of the experiments recorded in Table VI, namely, the determination of the acetone produced in two successive half-hour periods by perfusion of the liver with dog's blood containing sodium butyrate. The volume of blood used was proportional to the size of the animal, the animal's own blood alone being used. 350 c.c. of blood containing saline were used for a 6-8 kilo animal, and to this was added

TABLE VII. Acetone production in dogs' livers perfused with blood containing sodium butyrate.

Exp	Weight of liver gm		Fat %	Glycogen %	Acetone production	
					Period I	Period II
30	225	Control	1.9	1.15	66	86
31	235	do	4.3	0.15	62	66
32	275	Insulin present in Period II	11.0	0.10	99	96
33	295	do	2.4	0.10	139	139

1 grm. of butyric acid as the sodium salt in 10 c c of saline. At the beginning of the second period, 3 c c of the same solution and, in two of the experiments 20 units of insulin were added.

Owing to the larger amount of blood available duplicate estimations of acetone bodies were carried out in this series (Table VII)

These results confirm those on the cat's liver and show definitely that insulin does not affect the production of acetone from butyric acid in the perfused dog's liver

*Ratio of acetone bodies formed to butyric acid oxidised in the liver*

It was found possible to follow the rate of removal of butyric acid from the blood by determination of the residual butyric acid in 10 c c samples of blood taken at intervals during the perfusion

The blood is diluted and freed from protein by the same method as is adopted in the acetone determination by van Slyke's method. 100 c c of the filtrate are measured into a litre flask, made up to 160 c c with water and distilled at the rate of 110 c c in 30 minutes as in the Reichert-Wollner volatile acid determination. The total distillate is then titrated with  $N\ 20-N/30$  sodium hydroxide to phenol phthalein.

In order to determine the accuracy of the method, known quantities of butyric acid were added to the filtrate from normal blood, distilled, and titrated. The results are tabulated below

Butyric acid added mg	Butyric acid found mg
0.35	0.2 = 0.1
4.7	4.5 = 0.1
1.9	1.9 = 0.1
0.9	0.9 = 0.1

The estimation of small quantities is therefore accurate within the error of the titration ( $0.2\ c\ c\ N\ 20\ NaOH = 0.88\ mg$  butyric acid). The larger quantities of butyric acid are recoverable under these conditions to the extent of 96-98 p c

When butyric acid is added to the blood *before* precipitation of proteins a correction has to be introduced owing to adsorption of the acid on the protein precipitate. If the specified conditions of precipitation are adhered to the adsorption of butyric acid is constant at any given concentration but varies with the concentration. The following are the results obtained when known quantities of butyric acid were added to normal blood



Butyric acid added mg	Butyric acid found mg	Recovery %
13.5	12.5	93
9.0	8.3	92
4.5	4.1	91
2.25	1.8	80
9	5	56

A graph drawn from these results enables the true concentration of butyric acid to be calculated from the figure obtained by titration. When the amount of butyric acid distilled is greater than 4.5 mg, multiplication of the value found by the factor 1.08 gives the true value to within 2 p.c.

These figures were then applied to the determination of the amount of butyric acid removed from the perfusing blood, and compared with the amount of butyric acid calculated to give rise to the acetone formed during the perfusion. Since the latter figures depend on the total acetone estimation of van Slyke, which is an approximation arrived at by assuming that in blood the ratio of acetone present as acetone + acetoacetic acid to that present as  $\beta$ -oxybutyric acid is as 1 : 2, the variations in the percentage oxidation of butyric acid to acetone are probably ascribable to variations in the ratio acetone :  $\beta$ -oxybutyric acid. The results are tabulated below.

TABLE VIII.

Exp	Time of perfusion mins.	Butyric acid corresponding to acetone found mg	Total butyric acid removed mg	% conversion of butyric acid to acetone
58	80	270	257	105
59	80	186	202	92
60	85	142	150	94
61	80	158	209	74
27	80	288	453	63.5
28	110	382	380	100.5
29	145	233	329	67.5
62	100	347	500	69.5
63	80	337	441	76

The total acetone produced in control perfusions without added butyric acid has not been deducted, so that the figures in the last column represent the maximum oxidation of butyric acid to acetone, since it is impossible to say whether any of the acetone found is derived from substances other than butyric acid, i.e., whether the mean figure obtained in the control perfusions without butyric acid should be deducted from the total acetone in these experiments. It seems likely that in the presence of a substance so easily oxidised as butyric acid, the normal oxidative reactions would be partially or completely suppressed, hence that the

acetone found in the presence of butyric acid is derived entirely from butyric acid. This would mean that the oxidation of butyric acid in the liver results in the formation of acetone bodies to the extent of an average of 80 p c of the theoretical.

*The influence of fat and glycogen on acetone production in the liver* It appeared from the results of perfusions in which no butyric acid had been added to the blood, that the capacity of the liver to produce acetone bodies was very closely related to the fat content of the liver, and was affected also by the presence or absence of glycogen in the liver. These results are tabulated below.

TABLE IX Perfusions without added butyric acid.

Exp	Fat %	Glycogen %	Blood sugar %		Acetone produced mg per hour
			Beginning	End	
34	30	0.10	0.500	0.545	58.5
35	30	0.02	0.485	0.550	63
36	19	0.50	0.680	0.880	14.5
37	8	—	0.460	0.542	35
38	5	0.20	0.560	0.610	14
39	5	0.10	0.285	0.240	26
40	5	—	0.640	0.620	23.5
41	3	0.50	0.58	0.58	24
42	3	0.25	0.340	0.350	24.5
43	—	0.05	0.206	0.224	70
44	—	0.10	0.460	0.678	11
Dogs					
65	2.3	3.0	0.455	0.600	2
66	2.5	0.10	0.505	0.660	8.6
67	2.9	0.50	0.280	0.510	15.0
68	3.2	0.10	0.225	0.175	36

In Exps 35, 36, 38-42, insulin was added at the beginning of the perfusion. So far as can be seen—the large variation in acetone production making any comparison difficult—the insulin was without action. Regarded as a whole, the figures in Table IX show (i) that with a fat content of 5 p c or less, the acetone production tends to be small and relatively constant, (ii) with a high fat content of the liver the acetone production is increased, (iii) the effect of high glycogen content is to depress acetone production (Exps 36, 45). The figures previously given in Table I show that these generalisations apply also to the perfusions with pig's blood.

The amount of available glucose in the system, as indicated by the level of the blood sugar, which varied from 0.20-0.90 p c, appears to have no effect whatsoever on the acetone production.

*Carbohydrate metabolism of the perfused liver* It has been previously mentioned that insulin has no apparent action on glycogenolysis in the

liver The blood-sugar curves obtained during the perfusions with and without insulin are similar and no evidence has been obtained in these experiments that insulin tends to bring about a deposition of glycogen On the contrary the blood-sugar generally rises during the perfusion even when insulin is added and the residual glycogen, which, in 11 control experiments averaged 0.28 p.c., in 15 insulin experiments (of all types) averaged 0.23 p.c. In several experiments in which ice-cold saline was used preliminary to perfusion with blood, the blood-sugar fell slightly, but this fall was not increased by addition of insulin

In a large number of experiments the blood lactic acid was estimated and the slope of the lactic acid curve was in almost all cases in the opposite direction to that of the blood-sugar curve The absolute amounts of glucose and lactic acid appearing and disappearing were not great, however, nor were they equivalent, that of the glucose being always the greater

TABLE X. Changes in blood sugar and lactic acid during perfusion of liver

Exp	Time of perfusion mins	Blood sugar %		Lactic acid %	
		Beginning	End	Beginning	End
45	60	0.341	0.373	0.100	0.073
46	60	0.732	0.892	0.077	0.070
47	60	0.762	0.775	0.054	0.032
48	60	0.268	0.300	0.066	0.063
49	60	0.541	0.690	0.080	0.054
50	60	0.410	0.458	0.060	0.035
51	90	0.294	0.357	0.046	0.034
52	60	0.294	0.340	0.080	0.041
53	120	0.190	0.260	0.053	0.045
36	60	0.206	0.224	0.026	0.021
37	120	0.460	0.638	0.078	0.033
54	120	0.260	0.280	0.044	0.032
55	60	0.537	0.586	0.060	0.052
56	100	0.850	1.035	0.110	0.100
57	60	0.545	0.450	0.044	0.039
58	60	0.328	0.308	0.044	0.044
59	60	0.306	0.268	0.050	0.055
60	60	0.274	0.250	0.042	0.050
61	60	0.280	0.236	0.039	0.056

## DISCUSSION

The failure of insulin in these experiments to inhibit, as it does in diabetes, the formation of acetone bodies, is capable of explanation in two different ways The experiments show that the isolated liver perfused with blood to which no butyric acid has been added produces acetone bodies to a variable extent, the amount formed being dependent on the glycogen and fat content of the organ Glycogen inhibits acetone body formation and fat increases it Since the amounts of these two substances

in the liver under physiological conditions usually vary inversely, it is not possible to say that one or the other is the more potent factor. Several of the experiments however suggest strongly that it is the glycogen content which governs the formation of acetone bodies.

Insulin, according to the experiments of Frank, Hartman and Nothmann(13), Banting and others(14), causes a deposition of glycogen in the liver of the intact or depancreatized animal, and its failure in the perfusion experiments to affect the formation of acetone bodies may be due to the fact that it failed also to affect the glycogenic function, presumably because the nervous control was lacking. The other explanation would be that insulin does not effect the inhibition of the formation of acetone bodies in diabetes by an action on the liver but on some other organ or tissue and hence with the *isolated* liver no influence of insulin on acetone body formation might be expected. Because of the localisation of the production of acetone bodies in the liver, it is difficult to accept an explanation of this latter kind although it appears theoretically possible, and the authors incline therefore to the first view as being the more probable. If this be the true explanation, then the antiketogenic action of insulin in diabetes must be attributed to its ability to cause glycogen storage in the liver in the intact animal and not to any modification of the oxidative processes which fat undergoes.

Since there was an abundance of glucose in the blood during all the experiments, it appears that a supply of glucose alone does not affect the formation of acetone bodies, nor does glucose in the presence of insulin exercise any antiketogenic action, so that the hypothesis that it is some more reactive form of glucose formed under the influence of insulin which has antiketogenic properties, is also not borne out. The assumption that some form of chemical combination occurs between acetone bodies and carbohydrate, in order that the former may be oxidised has been made by Geelmuyden(16), Ringer(17), Woodyatt(18) and Shaffer(19). The experiments described in this paper do not give any evidence in favour of such views, for the presence of an abundance of glucose in the blood and the fact that glycogenolysis was taking place in most of the experiments do not appear to have influenced the formation of acetone bodies, either without the addition of sodium butyrate or in its presence. The fact that the amount of glycogen in the liver modifies the production of acetone bodies might be taken to mean that this carbohydrate is the one with which they must combine in order to be oxidised further, but this seems very unlikely on general grounds. A more reasonable interpretation of the antiketogenic action of carbo-

liver The blood-sugar curves obtained during the perfusions with and without insulin are similar and no evidence has been obtained in these experiments that insulin tends to bring about a deposition of glycogen On the contrary the blood-sugar generally rises during the perfusion even when insulin is added and the residual glycogen, which, in 11 control experiments averaged 0.28 p c, in 15 insulin experiments (of all types) averaged 0.23 p c In several experiments in which ice-cold saline was used preliminary to perfusion with blood, the blood-sugar fell slightly, but this fall was not increased by addition of insulin

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48	60	0.208	0.300	0.066	0.063
49	60	0.541	0.690	0.080	0.054
50	60	0.410	0.458	0.060	0.035
51	90	0.294	0.357	0.040	0.034
52	60	0.294	0.340	0.060	0.041
53	120	0.190	0.260	0.053	0.045
36	60	0.206	0.224	0.026	0.021
37	120	0.460	0.638	0.078	0.033
54	120	0.260	0.280	0.044	0.032
55	60	0.537	0.586	0.060	0.052
56	100	0.850	1.035	0.110	0.100
57	60	0.545	0.450	0.044	0.039
58	60	0.328	0.368	0.044	0.044
59	60	0.306	0.268	0.050	0.055
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# THE IMPULSES PRODUCED BY SENSORY NERVE- ENDINGS Part 4 Impulses from Pain Receptors

By E D ADRIAN

*(From the Physiological Laboratory, Cambridge)*

THE present investigation deals with the analysis of the impulses set up in cutaneous nerves by painful stimuli and compares the pain discharge with that of other receptors. The older view that pain resulted from excessive stimulation of any cutaneous receptor has been largely abandoned in favour of the idea of special pain receptors and conductors. The specific character of "pain" suggests that these receptors and conductors may differ considerably from those of the other types of cutaneous sensation. For instance, the "protopathic and epicritic" hypothesis of Head and Rivers demands that the nerve fibres concerned with pain must belong to a system phylogenetically distinct from those concerned with touch and the histological work of Ranson has added the view that the pain fibres may be non-medullated. Again, the pain receptor probably differs from many others in the absence of a capsule surrounding the axon termination and for this reason it has been suggested that the discharge of impulses from the pain endings may have some characteristic frequency or grouping of its own. These and similar possibilities can evidently be tested by recording the impulses produced in the afferent nerve fibres by a painful stimulus.

Preliminary observations<sup>(1)</sup> with the capillary electrometer and amplifier showed that it was possible to record the action currents set up in afferent nerves when the skin was pinched or pricked. The next step was to secure that not more than a few nerve fibres should be in action together so that each impulse might be recorded without interference. Apart from the use of nerves with very few fibres two general methods may be applied in any research of this character, namely (1) to restrict the stimulus to a very small area so that only a few receptors are stimulated, (2) to destroy or put out of action all but a few of the receptors or nerve fibres which will be affected by the stimulus. The former method is suited to the present case since a prick with a very fine needle will produce sensation which is clearly painful, but will scarcely stimulate

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*(From the Physiological Laboratory, Cambridge)*

THE present investigation deals with the analysis of the impulses set up in cutaneous nerves by painful stimuli and compares the pain discharge with that of other receptors. The older view that pain resulted from excessive stimulation of any cutaneous receptor has been largely abandoned in favour of the idea of special pain receptors and conductors. The specific character of "pain" suggests that these receptors and conductors may differ considerably from those of the other types of cutaneous sensation. For instance, the 'protopathic and epicritic' hypothesis of Head and Rivers demands that the nerve fibres concerned with pain must belong to a system phylogenetically distinct from those concerned with touch and the histological work of Ranson has added the view that the pain fibres may be non-medullated. Again the pain receptor probably differs from many others in the absence of a capsule surrounding the axon termination and for this reason it has been suggested that the discharge of impulses from the pain endings may have some characteristic frequency or grouping of its own. These and similar possibilities can evidently be tested by recording the impulses produced in the afferent nerve fibres by a painful stimulus.

Preliminary observations (1) with the capillary electrometer and amplifier showed that it was possible to record the action currents set up in afferent nerves when the skin was pinched or pricked. The next step was to secure that not more than a few nerve fibres should be in action together so that each impulse might be recorded without interference. Apart from the use of nerves with very few fibres two general methods may be applied in any research of this character, namely (1) to restrict the stimulus to a very small area so that only a few receptors are stimulated, (2) to destroy or put out of action all but a few of the receptors or nerve fibres which will be affected by the stimulus. The former method is suited to the present case since a prick with a very fine needle will produce sensation which is clearly painful, but will scarcely stimulate



a large number of pain endings. The ideal site for the stimulus would be a region sensitive to pain but not to touch, *e.g.* the cornea or the glans, but considerable technical difficulty would attach to working with these regions and it was decided to use a more convenient area of skin surface, bearing in mind that the stimulus might set up impulses in other receptors besides those of pain. Most of the experiments were made on the spinal (decapitated) cat using the preparation of the internal plantar nerve employed by Zotterman and the writer<sup>(2)</sup> for the study of impulses due to pressure. A few confirmatory experiments were made on the frog, these although much easier to carry out present the disadvantage that we know little about the character of the sensations or sensation provokable from the frog's skin.

*Stimulating apparatus.* Two methods of stimulation were used. In both a prick was delivered by a fine needle (usually a gilt "entomological pin") clamped in the end of a vulcanite rod (*N*, Fig 1). In the

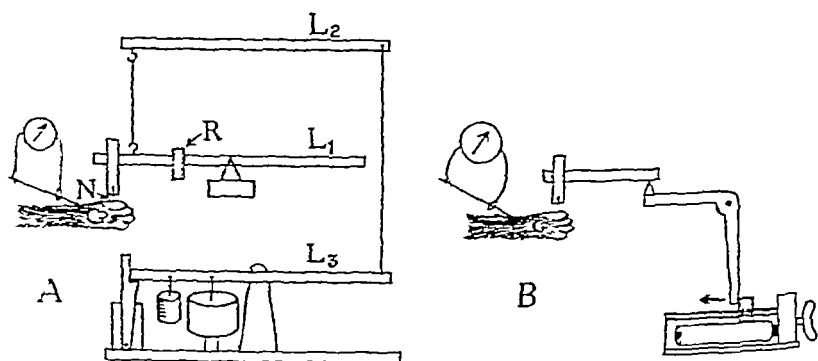


Fig 1 Stimulating apparatus. *A* Constant weight on needle. *B* Needle pressed slowly through skin.

first method the rod is fixed at the end of a lever  $L_1$ , pivoted at its midpoint and carrying a rider  $R$  which can be moved so that the weight on the needle varies from 3 to 99 gm. The lever  $L_1$  is connected by a string to another lever  $L_2$  which in turn is connected to  $L_3$ , an arm which can be moved up or down at a constant rate by a weight acting against the resistance of an oil dash pot. In the resting position  $L_1$  is held so that the point of the needle is 1 cm. above the skin. When  $L_3$  is released the needle is lowered smoothly at a rate of about 2 cm. per second and comes to rest on the skin with a weight determined by the position of the rider. When this apparatus is tested on the palmar surface of the finger tip a momentary sensation of touch is all that is produced when the weight on

the needle is only 3 grm. When it exceeds 30 grm. a sharp prick is felt and the duration and intensity of the pain increases with greater weights. In regions where the skin is more delicate pain may be felt with a much smaller weight than 30 grm. The advantage of the method lies in the fact that the stimulus can be graded to some extent by altering the weight, but it has the disadvantage of producing its greatest effect at the moment of contact, when touch as well as pain receptors may be stimulated.

In the second method (Fig. 1 *B*) the needle is pushed slowly into the skin. The rod holding the needle is fixed to a lever which is coupled to a long arm the end of which presses against the sound box carrier of a clockwork driven phonograph. When the phonograph is set in motion the carrier is moved forward slowly by a fine screw and the needle point moves downwards at a rate of 3–5 mm. per minute. In the resting position the needle is set so that its point is just touching the skin. When the finger tip is tested, an ill-defined sensation of contact is experienced when the clockwork is started but this soon gives place to one of pain which becomes more and more intense as the needle is driven deeper into the skin. The pain diminishes slowly after the movement is stopped. This method has the advantage of giving a gradually increasing stimulus and of giving one which is bound in the end to be painful however tough the skin may be.

#### A. *Experiments on cats*

The experiments on cats will be described first. The animal was decapitated under chloroform anaesthesia and the hind limbs were immobilised by nerve section. The medial branch of the internal plantar nerve was dissected out as far down as the point where it is crossed by a small blood vessel about 1 cm. from the end of the toe. The upper end of the nerve was cut just below its entry into the main trunk of the internal plantar. A diagram of the nerves concerned is given in Part 3 of the present series. The nerve supplies the skin on the medial side of the inner toe, the skin on the side of the toe pad is hairless and not very tough and it was in this region that the stimulus was applied. The hairs in the neighbourhood were all cut short.

The animal was placed on an insulated stand inside the metal preparation box and the leg was fixed to a smaller stand carrying the electrodes. The lateral side of the inner toe was supported by a rigid platform to prevent it moving under the pressure of the needle. The interior of the preparation box was warmed, the nerve was constantly irrigated with Ringer at 37° C. and between the observations it was taken off the

electrodes and covered with lint soaked in Ringer and kept warm by a small carbon lamp 2-3 cm away. With these precautions the nerve usually remains in good condition for more than an hour.

Twelve animals were used and in all of them occasional impulses passed up the nerve in the absence of any kind of stimulation. The frequency of this "resting" discharge is low (5-40 per second) when the preparation has been set up for ten minutes or more, but may be very high in records taken immediately after the dissection is finished. Fig 2 shows the confused medley of action currents obtained just after

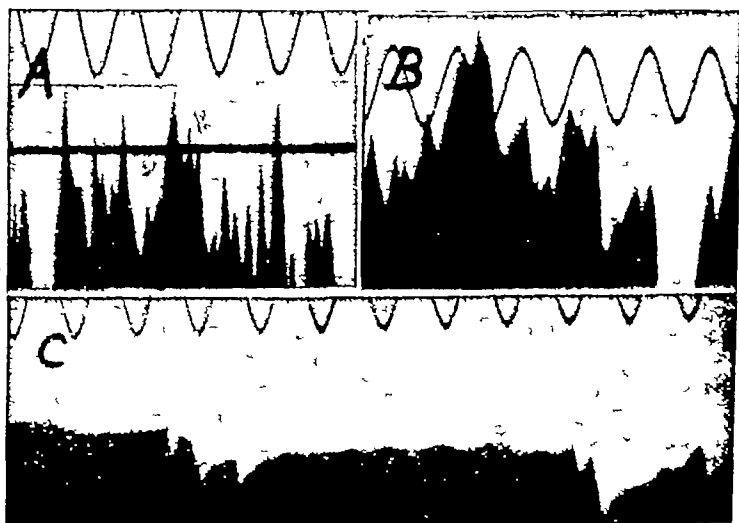


Fig 2 Resting' discharge from medial branch of Internal Plantar nerve (cat) A Exp 4 Preparation just set up. No stimulation. B Exp 1 Preparation just set up. No stimulation. C Exp 1 Ten minutes later

the preparation is set up and the much lower frequency ten minutes later. This initial discharge is presumably due either to pain impulses arising from damage to the skin in the region supplied by the nerve or to the stimulation of other receptors (*e.g.* hairs, temperature, etc.) by the new conditions to which the foot is exposed. After the initial discharge has subsided the resting frequency usually remains at constant value or declines progressively. It has never shown a marked rise in the absence of stimulation.

*Results* The film records given in Fig 3 (Exp 2) show the nature of the discharge produced by the first method of stimulation (needle

lowered on to skin and allowed to remain there with constant pressure) The dark signal line merely records the movement of the lever  $L_2$  and

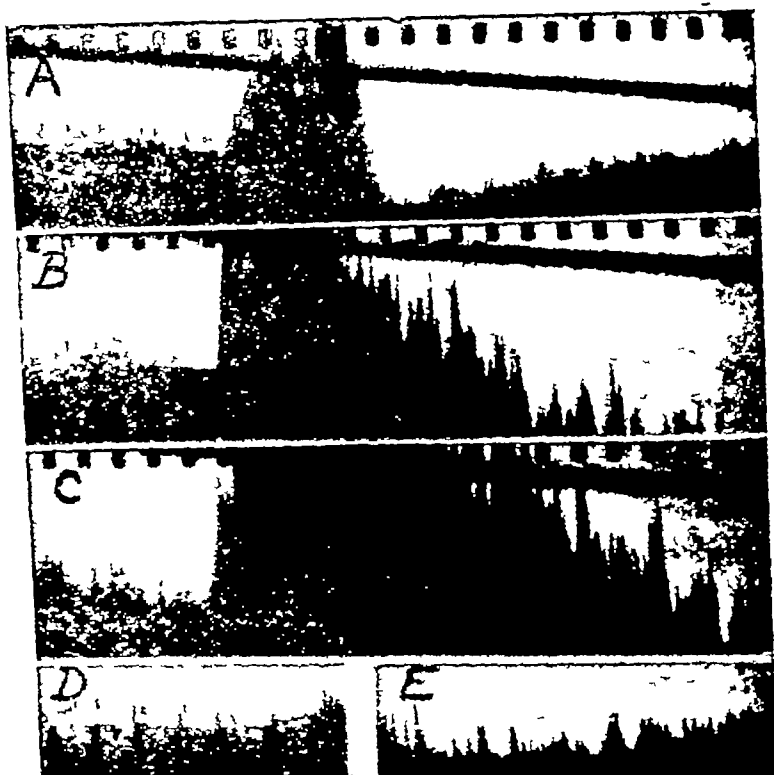


Fig 3 Exp 2 (cat) Stimulation by first method (constant weight) Time marker gives  $\frac{1}{20}$  second intervals. A Weight on needle 3 grm. B Weight on needle 43 grm. C Weight on needle 99 grm D Pesting discharge before stimulation (same record as C) E Same record as C 18 seconds after beginning of stimulation.

does not give the actual moment at which the needle touches the skin, but this last is very clearly signalled by the sudden outburst of impulses in the nerve There is a resting discharge at a rather higher frequency than usual (about 40 a second) and in the top record, where the weight on the needle is only 3 grm., the contact of the needle with the skin gives an increased frequency of discharge which only lasts for 15 second

When the weight is increased to 43 and 99 grm (Fig 3 B and C) a persistent discharge is set up, and Fig 3 E shows that the frequency is still considerably above normal after the needle has remained in position for 18 seconds At the height of the discharge there is great overlapping

between impulses in different fibres and the total frequency is difficult to state correctly, but within a second after the moment of contact the overlapping is much less and the frequency has fallen to 150 per second. The decline in frequency is shown in Fig 4 which gives the total fre-

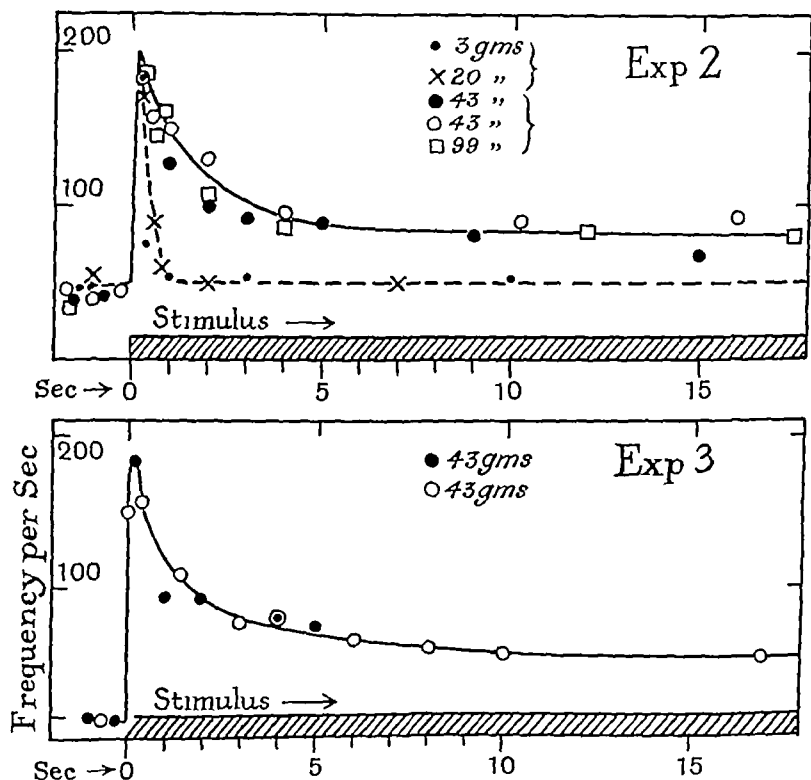


Fig 4. Decline in frequency of discharge with constant stimulus

quencies per second in Exps 2 and 3 with this form of stimulation. The value given for the maximal frequency (200 per second) may be too low but this figure was not exceeded in records on fast moving plates made within  $\frac{1}{2}$  second of the moment of contact.

In Exp 2, it will be seen that the curves fall into two groups. When the weight on the needle is 3 gm or 20 gm the discharge has returned to its initial ("resting") frequency within a second, when the weight is 43 or 99 gm the frequency declines much more slowly and has not returned to the initial value in 20 seconds or more. This grouping is probably related to the fact that on the finger the smaller weights give

only a momentary sensation of touch whereas the larger give a more persistent sensation of pain. Stimuli with 43 or 99 grm cause a brisk flexion in the spinal cat with nerves intact and with 99 grm the point of the needle was found to have penetrated the skin 1 mm or more. Thus the majority of the impulses set up by the strong stimuli after the first second are probably due to the stimulation of pain receptors.

Since the total frequency after one second of stimulation is not more than 200 per second at most, the number of impulses discharged in a second in each nerve fibre cannot be higher than this. The impulses might perhaps be discharged at a much higher frequency in very short bursts with pauses in between, but there is no indication of this in records taken on rapidly moving plates. An example of such records is given in Fig 5*A*, it will be seen that the impulses occur without characteristic spacing

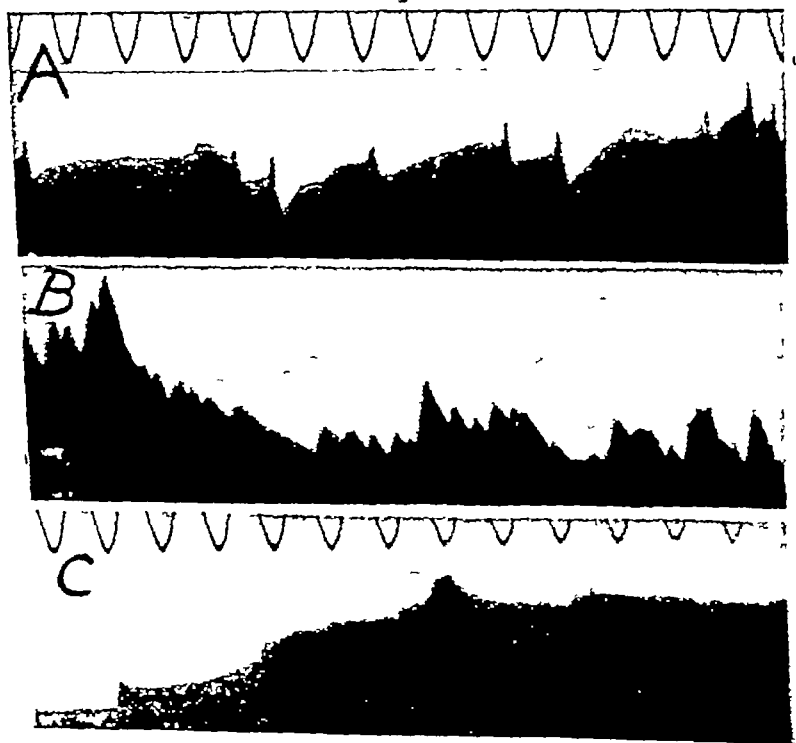


Fig 5 Plate records (cat) Time marker gives 0.1 second intervals. *A* Exp. 4. Stimulation by first method. 34 grm. weight for half second. Diphasic action currents. *B* Exp 5 Stimulation by second method. Needle driven in slowly for 20 seconds. Monophasic action currents. *C* Exp 5 Before stimulation.

More decisive information is given by the second method of stimulation in which the needle is pushed slowly through the skin, in this case there can be no question but that most of the impulses arise from pain receptors Fig 6 (Exp 5) shows the evolution of the discharge under

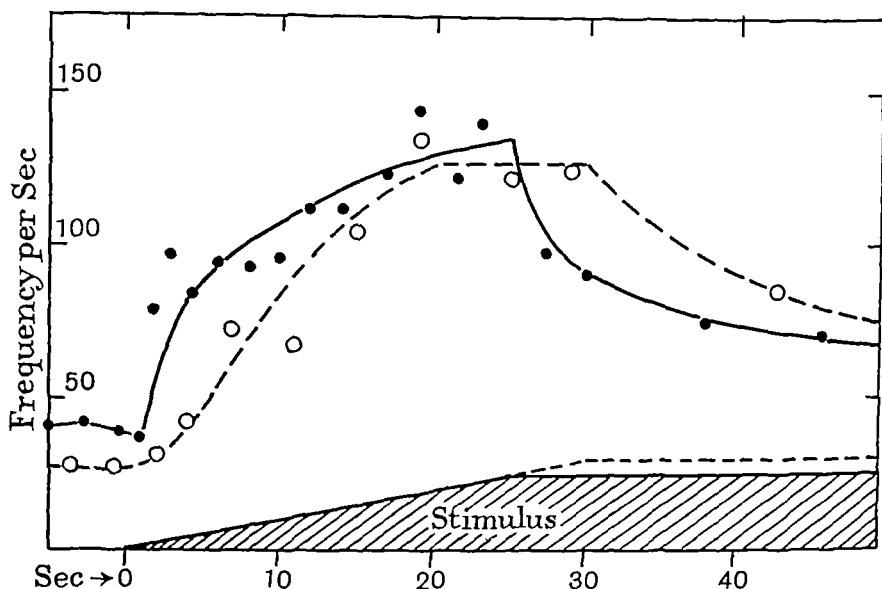


Fig 6 Exp 5 Gradual increase in frequency as needle is driven into skin.

this method, the curves being constructed from two continuous film records. The frequency of discharge rises gradually from the resting value and after the downward movement of the needle has proceeded for 20 seconds the frequency reaches a more or less steady value of about 140 per second. It is difficult to count this in the film records owing to the crowding together of the impulses, and records taken on rapidly moving plates at 20 seconds from the beginning of stimulation gave a higher frequency, 180-200 per second. One such record is shown in Fig 5 B. Since the needle has penetrated 5 to 1 mm into the skin in 20 seconds and since with the finger-tip under like circumstances acute pain is reached at that stage, the majority of the impulses shown in Fig 5 B are certainly due to pain receptors. Indeed the curves in Figs 4 and 6 giving the rise and decline in the frequency of the impulses are in surprisingly close agreement with the rise and decline in the pain sensation produced by the two forms of stimulation applied to the tip of the finger.

*Time relations of pain impulses* The view that pain impulses are carried by nerve fibres different in character from those subserving other species of sense suggests that possibly the time relations of the individual impulse may be different. In particular, if the pain fibre is non-medullated the rise and decline of the "pain" impulse should be much the slower. The time relations of a typical pain impulse may be determined from an analysis of the monophasic responses recorded when the needle is thrust slowly into the skin. Fig 5 B gives a record of this kind and an analysis of two of the impulses is shown on an extended time scale in Fig 7. For comparison with it an analysis is given of mono-

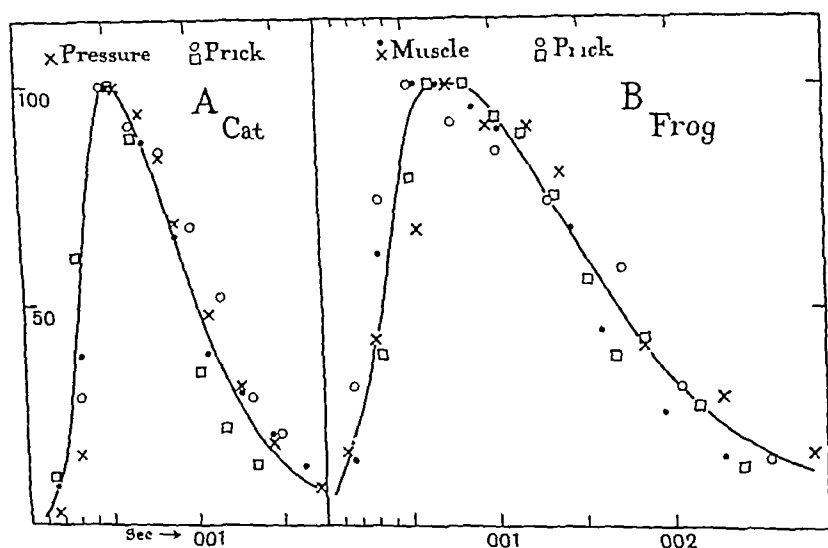


Fig 7 Time relations of impulses produced by different receptors in the frog and cat. All monophasic. Temperature in frog experiments between 12 and 14° C

phasic impulses taken at random from the records of Zotterman and the writer with the same preparation stimulated by pressure. The pressure in this case was 200 grm on a disc 11 mm in diameter and it is very unlikely that this would produce a sensation of pain. Yet the two impulses clearly agree within the limits of experimental error and their duration is much shorter than one would expect from non-medullated nerve fibres. It is difficult to assess the limits of error in these measurements since so much depends on the accuracy of the analysis, but the time relations shown in the figure agree fairly well with those given by



Erlanger and Gasser(3) using the cathode ray oscillograph. The latter have shown(4) that there may be considerable differences in the rates of conduction in different afferent fibres although the duration of the action current is much the same in all. It is therefore quite possible that the pain fibres may possess a distinctive conduction rate in spite of the agreement in the action currents in Fig 7.

As may be seen from the photographs (Fig 5), there is very little variation in the duration of different impulses. In certain records there are occasional excursions with a duration about twice as long and an amplitude (when analysed) about one-fifth as great as that of the usual impulses. These have been met with in records of moderate pressure as well as in those of pain and they are not more frequent with painful stimuli. When present their frequency is low (*e.g.* 20 per second). They can scarcely be due to impulses travelling in incompletely recovered nerve since they may occur by themselves in a quiet period. It is possible that they may be identical with the "delta" process of Erlanger, Bishop and Gasser(5). Whatever they are there is no reason to suppose that they are the typical product of the pain receptors in the skin.

*Grouping of impulses* As far as the grouping of impulses is concerned there is nothing to distinguish a discharge of pain impulses from that of impulses due to moderate pressure. In both the total frequency will depend on the number of receptors in action, but if the area covered by the stimulus is such that the total frequency is about 150–250 per second the records would be indistinguishable. This may be seen from a comparison of the present figures with those given in Part 3. In both the impulses are more or less evenly spaced and show little variation in amplitude apart from the larger excursions made up of two or three impulses occurring simultaneously. When two impulses are very close to one another the second is never markedly smaller than the first as it would be if both occurred in the same fibre and there is no sign that the discharge ever takes the form of a rapid series of impulses in each fibre followed by a pause and then another rapid series in the same fibre. Regular rhythms have been observed in two only of the experiments. In one (Exp 4) a prick of 20 gm weight applied for 1 second gave a regular series with a period of 0.3 per second and the same stimulus applied for 5 seconds gave a regular period of 0.37 per second. Both records contain other impulses which cannot be grouped into regular series. In the other experiment (Exp 6) there were periods of 0.29 and 0.4 second in the same record taken when the needle had been resting

on the skin with a weight of 43 grm for 20 seconds. In the same preparation a weight of 8 grm for 3 seconds gave a regular discharge with a period of 0.163 second. This result is interesting as showing that a stimulus which is probably non-painful may evoke a higher rate of discharge than one probably painful. The total frequency is however so low, even when the needle is being pushed slowly through the skin, that there can be no doubt that the discharge of impulses produced by a painful stimulus is not characterised by a specially high frequency in each nerve fibre. Judged both by the total frequency in the nerve and the occasional regular rhythms, the usual frequencies in the individual fibre correspond with that found by Zotterman and the writer for the pressure receptors, and are of the same order as those found by them for the receptors in the frog's muscle.

In most of the records it has not been possible to trace any regular rhythms, but here, as in the case of pressure stimuli, a sufficient explanation may lie in the fact that a single nerve fibre leads from a number of pain endings which may interfere with the activity of one of another. Owing to the absence of regular rhythms it is impossible to say how far the frequency in each nerve fibre varies with the intensity of the stimulus. When the pin is pushed slowly through the skin the rise in total frequency is no doubt due in part to an increase in the number of fibres in action, though the fairly uniform distribution of the impulses (e.g. in Fig. 5B) suggests that each fibre is maintaining a high rate of discharge.

### B *Experiments on Frogs*

For many reasons it is very much easier to carry out experiments of this kind on the frog instead of the cat. The nerves are more easily dissected out, they need not be kept at 37° C. and the whole routine of the experiment is much simpler. A difficulty lies however in interpreting the results. In the case of the frog, it is much less clearly possible for the observer to discriminate between stimuli applied to its skin as to their being painful or painless. Further, the skin-receptors of the frog are morphologically much less diverse than those of mammals<sup>1</sup>. Nevertheless, it seems unlikely that the nerve impulses evolved by pricking

<sup>1</sup> According to Gaupp (6) there are only two forms of sensory ending: one consists of an arborisation of naked axis cylinders about the cells of the epidermis; the other is more highly differentiated, occurs only in certain parts of the skin surface, and consists of a small group of cells in the corium surrounding the terminations of a medullated nerve fibre. The latter is generally regarded as an organ of pressure or touch ("Macula tactus").

the frog's skin with a needle, whether or no they give rise to a reaction truly describable as "pain," differ considerably from the pain impulses in mammals

The nerve employed in the present experiments was the superficial branch of the tibial nerve. This sends a branch to the skin on the medial aspect of the leg a short distance above the ankle. All other branches were divided and the nerve was cut just below the knee. The skin was so dissected that an area about 1 cm square about the point of entry of

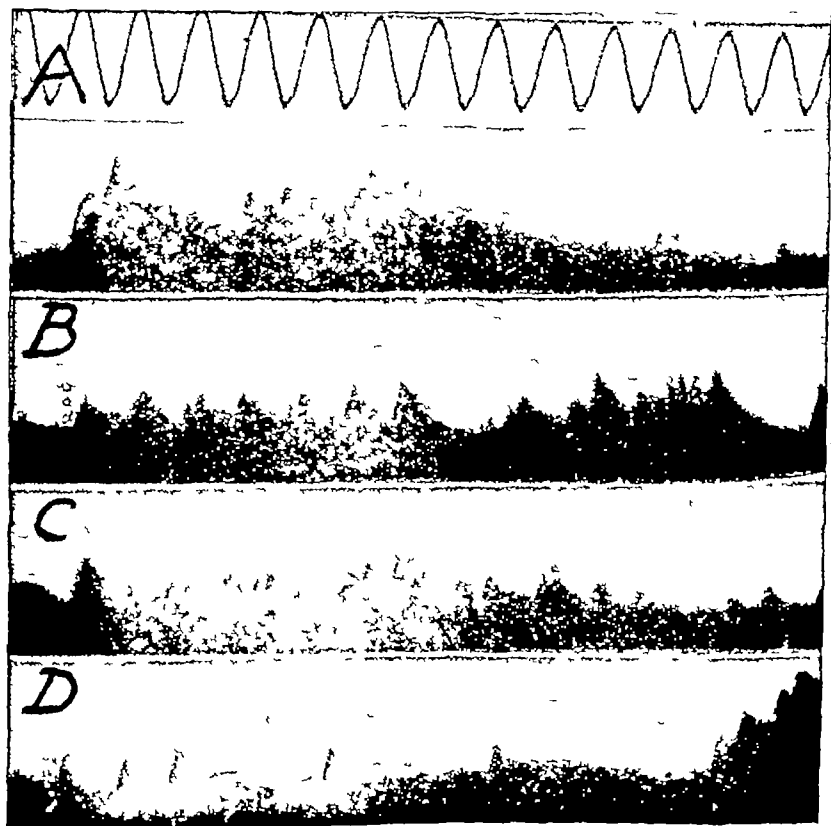


Fig 8 Plate records from superficial branch of tibial nerve (frog) Time marker gives 0.1 second intervals. A, B and C Exp 14 Constant weight on needle. Moment of stimulation coincides with release of plate. A Weight  $\frac{1}{4}$  gm. The record shows almost the entire discharge. B Weight 10 gm. Regular rhythm in first part of record. C Weight 25 gm. D Exp 12 Second method. Needle driven through skin for 15 seconds

the nerve could be laid with the subcutaneous surface downwards on a pad of filter paper soaked in Ringer's fluid. In three experiments the skin and the nerve were removed completely from the frog, in three others one side of the skin was left attached to the leg.

When the nerve is connected with the amplifier and electrometer impulses are usually completely absent unless the skin be stimulated. At most there may be a discharge of two or three a second. The results of stimulating by the first method (constant stimulus) are shown in the records in Figs 8 and 9 and in the curves in Fig 10 (Exps 9 and 10). Four curves are given from two different frogs and it will be seen that they agree very closely. The maximum frequency is just under 150 per second and the records in Figs 8 and 9 show the absence of any characteristic grouping of impulses.

Records with the second method of stimulation (needle slowly penetrating the skin) are also given in Figs 8 and 9 and the gradual rise in frequency is shown in the curve in Fig 10 (Exp 11). Apart from the absence of a resting discharge, the lower total frequency and the more



Fig 9 Film records (frog) Time marker gives  $\frac{1}{10}$  second intervals. 1 and B Exp 10 Constant weight on needle (4.5 gm) A Stimulus begins B 3.5 seconds later Frequency declining C, D and E Exp 11 Needle driven slowly through skin C 4 seconds after clockwork started D 9 seconds E 18 seconds Frequency of discharge gradually increasing

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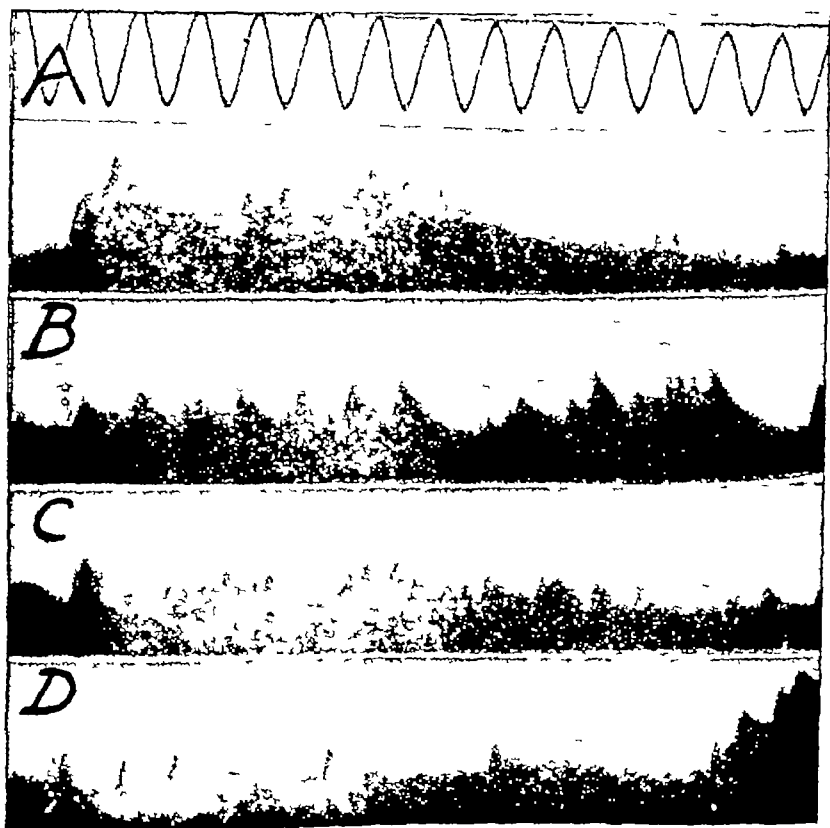


Fig 8 Plate records from superficial branch of tibial nerve (frog) Time marker gives 0.1 second intervals. A B and C Exp 14 Constant weight on needle. Moment of stimulation coincides with release of plate. A Weight  $\frac{1}{2}$  gm. The record shows almost the entire discharge. B Weight 10 gm. Regular rhythm in first part of record. C Weight 25 gm. D Exp 12 Second method. Needle driven through skin for 15 seconds.

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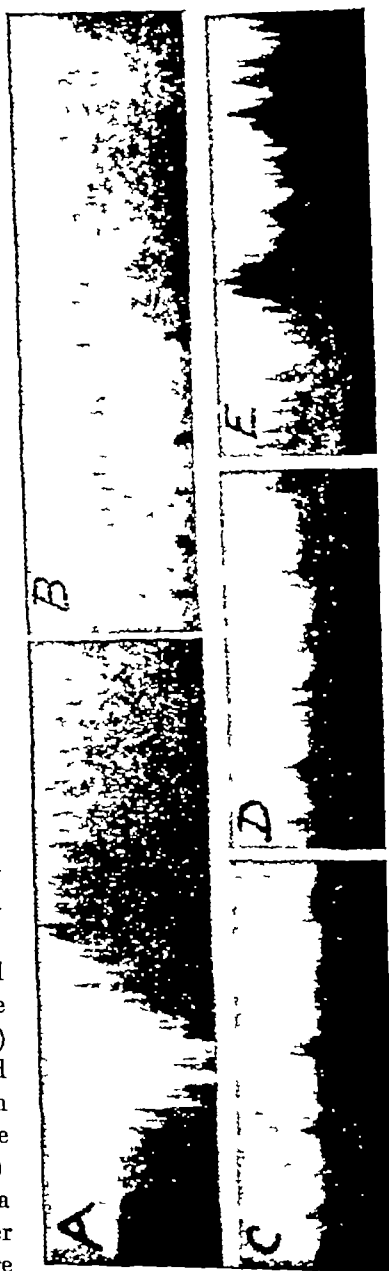


Fig 9 Film records (frog) Time marker gives 20 second intervals 1 and B 1 xp 10 Constant weight on needle (13 gram)  
 A Stimulus begins B 31 seconds later Frequency declining C, D and E 1 xp 11 Needle driven slowly through skin  
 C 4 seconds after clockwork started D 9 seconds E 18 seconds Frequency of discharge gradually increasing

rapid decline, these results are in complete agreement with those obtained in the cat

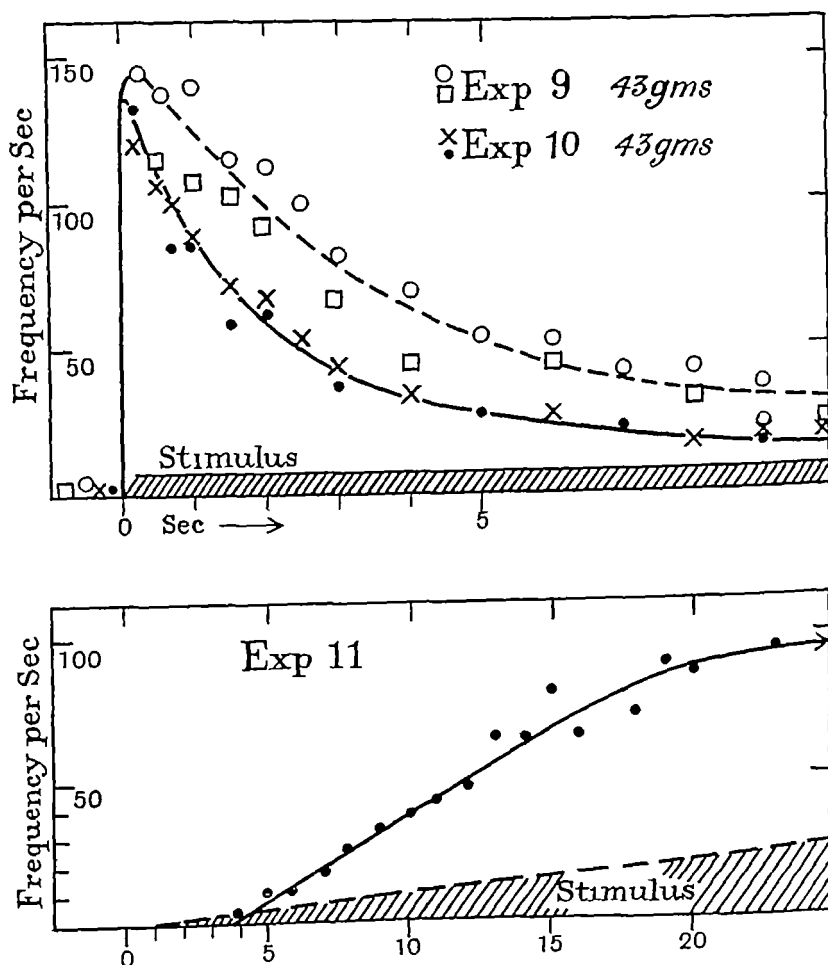


Fig 10 Impulses from frog's skin Decline in frequency with constant stimulus and gradual increase with increasing stimulus

An analysis of typical monophasic impulses is given in Fig 7 side by side with an analysis from the records of Zotterman and the writer of the afferent nerve impulses produced by a tension of  $\frac{1}{2}$  grm on the frog's sterno-cutaneous muscle. The duration of the impulses is naturally longer than in the mammalian nerve but there is again no difference between the impulses set up in the two different kinds of receptor

As in the records from the cat, regular rhythms are very uncommon. One with a frequency of 100 per second occurs in the first part of Fig 8 B, but it cannot be traced in the latter part of the record.

*Adaptation* The curves in Fig 10 show the decline in frequency when the needle remains in position with a load of 43 grm. This decline is much more rapid than that found for the muscle receptors of the frog stimulated by constant tension (cf Part 2). But the rate of adaptation, i.e. the decline in the effectiveness of the stimulus, must be even greater than is indicated in Fig 10. This appears when we study the effect of altering the strength of the stimulus. In the frog's muscle receptors an increase in tension produces an increased frequency of discharge and the rate of decline of the frequency when the tension is steady is always much the same. On the other hand, in the frog's cutaneous nerves, if the load on the needle is reduced from 43 grm to  $\frac{3}{4}$  grm the maximum frequency of the discharge is scarcely affected but the rate of decline becomes so rapid that the whole discharge is over in  $\frac{1}{2}$  second or less. This may be seen in Fig 8, where A is almost the entire discharge produced by a weight of  $\frac{3}{4}$  grm on the needle and C is the initial part of one lasting 3-4 seconds produced by a weight of 25 grm. The same result is shown in Table I, which gives the number of impulses counted in successive periods in another experiment made with continuous film records.

TABLE I.

Exp 13		Load on needle		
		43 grm.	4 grm	$\frac{3}{4}$ grm
Period		Number of impulses in successive periods		
Rest	- 1-0 sec.	0	0	0
Stimulus—0	- 05	7	7	8
	05- 1	7	4	6
	1 - 15	5	3	3
	15- 2	5	2	0
	2 - 25	7	3	0
	25- 3	4	2	0
	3 - 35	5	1	0
	35- 4	7	1	0
	4 - 45	3	2	0
	45- 5	2	2	1 (*)
	5 - 75	14	0	0
	75-1 0	15	0	0
	1 0 -2 0	31	2	0
	2 0 -3 0	25	0	1

Since the initial frequencies are so closely similar it seems unlikely that the longer discharge is maintained by additional receptors brought



into play by the stronger stimulus and having a slower rate of adaptation. Should that be so, the additional receptors must be credited with a latent period of 1 second or more, since otherwise the stronger stimulus would give a higher frequency during the first 1 second. It is more probable that we are dealing throughout with a group of receptors which adapt so rapidly that a  $\frac{3}{4}$  grm stimulus, though initially strong enough to produce the maximum rate of discharge, has become completely ineffective within 2 second, although a much stronger stimulus remains above the threshold value for a longer time.

This raises several points of interest. In the first place the receptor with which we are dealing is almost certainly the free intra-epithelial nerve ending, since the maculae tactus are very far apart in this region. It was pointed out by Sherrington(7) that the free nerve ending provides a suitable "pain" receptor since, *inter alia*, it may be expected to respond, like a nerve fibre, to any form of stimulus which is sufficiently strong. It is interesting, therefore, to find that the pain receptors adapt themselves to a constant stimulus at a rate comparable to that of a nerve fibre and much more rapid than that of a complex end organ like a muscle spindle. Although the time relations are not the same, the present records with different weights on the needle bear a close resemblance to Garten's records(8) of the multiple discharges produced by a constant current in a nerve trunk.

The varying duration of the discharge is important from another point of view. A prick of 43 grm undoubtedly provokes a much greater motor response in a spinal frog than does one of  $\frac{3}{4}$  grm. The maximal frequencies of the discharges are the same, and therefore the change in the response cannot depend on the frequency alone. If we make the assumption (discussed above) that the stronger stimulus does not bring any fresh receptors into play, the greater response must be due simply to the greater duration of the discharge. If this is so, the pain reaction must be determined by the summation of a number of impulses in the central nervous system and the very brief discharge might be inadequate to excite the appropriate arcs. It is possible that there are other paths open to a brief discharge, and in this way the skin receptors in the frog may conceivably serve a double purpose, calling up "touch reactions" when the stimulus is very weak and "pain reactions" when the discharge has a sufficient momentum to bring the pain mechanism into play.

Whether there is really any qualitative difference in the reactions of the frog to a very light and a very heavy prick it is hard to say, but

it is worth enquiring whether the skin receptors of the cat may not serve in the same way for touch and pain reactions according to the varying duration of the discharge set up. In the cat's pad a light touch with the needle point produces a very brief discharge (cf Fig 3)<sup>1</sup> and in man it may give a momentary sensation of contact. There can be little doubt that most sensations of touch are due to specific receptors, but it has long been known that the terminal organs anatomically known as "touch-corpuses" are far too sparse to provide of themselves the cutaneous sense of touch (contact). The intra-epithelial nerve endings could scarcely serve for lasting sensations of contact as well as for pain, but it seems quite possible that they may account for momentary sensations which are not painful.

### DISCUSSION

The foregoing analysis has shown that the discharge of the pain receptors differs very little from that of other receptors. The range of frequency in each nerve fibre is apparently much the same, the time relations of the individual impulses are the same and the impulses show no characteristic grouping. The only feature which is at all characteristic is the relation between the strength of the stimulus and the duration of the discharge set up. If we grant that the same receptor may give a very brief discharge with a weak stimulus and a much longer one when the stimulus is stronger, it seems likely that the character of the reaction is determined by the duration of the discharge, and it is conceivable that a momentary stimulation of the pain receptor may sometimes evoke a sensation which is not painful. The suggestion that a summation of impulses must occur before the pain reaction is aroused would provide an explanation for the supposed longer latency of the pain sensation as compared with other sensations. On the other hand, considerable difficulties are introduced by the fact that a single induction shock to a nerve trunk can give rise to pain. Evidently there are some pain fibres which have immediate access to the central mechanism of the pain reaction without the necessity for temporal summation, or else we must suppose that the simultaneous arrival of a number of impulses in different fibres is as effective as their successive arrival in one fibre.

Apart from these speculations it is clear that we must accept the established view of specific pain fibres which produce their effects in

<sup>1</sup> But it is noteworthy that in the cat a pain discharge may continue for a long time (30 seconds or more) with a constant stimulus if the needle has penetrated the skin. Possibly the rate of adaptation is slower for the endings which are more deeply situated.

virtue of their central connections and not in virtue of a characteristic type of impulse or arrangement of discharge

It is particularly interesting to find that the maximum frequency of the discharge in each nerve fibre is much the same for all the receptors so far investigated, namely about 150 per second in the cat and 100 per second in the frog. This suggests that the limits of frequency are not determined by the structure of the particular end organ but must depend on something common to all end organs, *e.g.* the properties of the naked axis cylinder. On the other hand, the rate of adaptation certainly varies widely as between one type and another of receptive organ.

### CONCLUSIONS

1 Impulses produced in cutaneous nerves by painful stimuli to the skin have been recorded in the cat and in the frog. By the use of a sharply localised stimulus (the prick of a fine needle) the number of fibres in action can be restricted so that individual impulses can be recorded without interference.

2 With a constant stimulus (the needle pressing with a constant weight on the skin) the frequency of the discharge declines rapidly from its initial, maximum value. With an increasing stimulus (the needle slowly driven through the skin) the frequency rises gradually and declines again when the movement is stopped. The intensity of the pain sensation in man follows much the same course.

3 The impulses produced by the pain receptors have the same time relations as those produced by pressure receptors (cat) and muscle receptors (frog). The frequency of discharge in each fibre seems to cover the same range and there is nothing characteristic in the grouping of the impulses.

4 The only distinction which can be drawn between the discharge of the pain receptor and that of other receptors lies in the very brief duration of the discharge with weak stimuli. It is suggested that these brief discharges may not be adequate to evoke the pain response but may serve instead for momentary sensations of contact, the development of the pain response depending on the summation of impulses in the central nervous system.

5 Since the range of frequency in each fibre is much the same for all the receptors investigated, it is presumably determined by some common factor such as the properties of the naked axis cylinder, and not by the particular structure of the end organ.

The expenses of this research were defrayed in part by a grant from the Government Grants Committee of the Royal Society.

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# REVERSAL OF THE ACTION OF ADRENALINE

By B A McSWINEY AND G L BROWN

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## INTRODUCTION

In a previous paper(1) we have been able to show that adrenaline may have either an augmentor or an inhibitory action on excised strips of muscle of certain regions of the rabbit's stomach. It had an inhibitory action on the pyloric sphincter, antrum and cardiac sphincter, and an augmentor action on the lower body and the circular fibres of the upper body and fundus. On strips taken from the longitudinal fibres of the upper body and fundus, it had in some preparations an augmentor, in others an inhibitor action. If an augmentor effect was elicited, addition of pilocarpine reversed the action of adrenaline, subsequent doses of adrenaline causing not a contraction, but a pronounced relaxation. These results suggest that the condition of "tonus" of the preparations determined whether adrenaline had an augmentor or an inhibitor effect.

Many references are found in the literature to the reversed action of adrenaline on smooth muscle. Cannon and Lyman(2) have obtained depressor effects with adrenaline, when the blood-pressure was high, a phenomenon which Dale and Richards(3) attributed to capillary dilatation. Kolm and Pick(4) demonstrated a reversal of the action of adrenaline on the heart by giving the drug subsequently to acetyl choline, but as the effect was abolished by atropine, they believed it to be due to adrenaline acting on the parasympathetic endings. Similar effects have been obtained on the heart and blood vessels by perfusing with fluids of varying pH and salt content. Cow(5) has recorded a reversal of the action of adrenaline in surviving strips of the guinea-pig's uterus, after the tissue had been "sensitised" by pituitrin.

It appeared desirable to investigate further the reversal of the action of adrenaline obtained after pilocarpine and to determine under what conditions it may occur. The shortening of the muscle fibres obtained by the addition of pilocarpine would appear to predispose to an inhibitor response with adrenaline, but the possibility of a chemical interaction between pilocarpine and adrenaline had first to be excluded. To show

the relationship between the length of the muscle fibre and the action of adrenaline, we have first studied the effect of other stimulating drugs, choline, histamine, pituitrin and barium chloride, on the reaction of the tissue to adrenaline

*Method* Rabbits, cats and dogs have been used in these experiments. Strips were excised from the stomach and suspended in Tyrode's solution at 38° C, through which a constant stream of oxygen was passed. The apparatus was similar to that previously described, except that the inner chamber was made of glass. 1 c.c. of the drug solution was added to 250 c.c. of Tyrode's solution in the chamber giving a dilution of 1:250,000. A 1:1000 solution of adrenaline (Parke Davis) was employed. Time intervals on the tracings equal 60 seconds.

### EXPERIMENTS

1 *Reversal by pilocarpine* Pilocarpine brings about reversal in the longitudinal strips from the fundus and upper body of the rabbit, a typical record of this action being shown in Fig 3. In many experiments, interesting mixed effects were obtained, the augmentor response being preceded by a slight inhibition. After pilocarpine the preliminary inhibition becomes the predominant feature and is followed by a slight augmentor action. In some experiments the pilocarpine did not cause complete reversal, simply converting a pure augmentor effect into an augmentor response preceded by a slight inhibition. In the other preparations of the rabbit's stomach, i.e. the lower body and circular fibres of the upper body, we were unable to obtain a definite reversal, the contraction produced by adrenaline being superimposed upon that caused by the pilocarpine. The same persistence of the augmentor response was observed by us in the cardia of the cat and dog (6).

2 *Reversal by other drugs (a) Choline* Doses of 40 mg. of choline chloride were used, as preliminary experiments had shown that this amount of the drug was approximately equivalent to 1 mg. of pilocarpine. The effect of choline on these preparations resembled closely that of pilocarpine. In the rabbit's fundus and upper body, addition of the drug was followed by a marked permanent shortening of the muscle fibres, but rhythmic contractions were little changed. In the lower body, rhythmic contractions were much augmented and there was slight permanent shortening. Choline was found to reverse the response to adrenaline in those regions in which pilocarpine had this effect. Choline, however, differed from pilocarpine as the relaxation produced by adrenaline seemed to be permanent, the muscle fibres not returning to

their former length. In many instances a complete reversal was not obtained, the preliminary inhibition being followed by an augmentor effect. Reversal was not obtained from strips of the lower body, the circular fibres of the body, or the cardia of the cat and dog.

(b) *Histamine* Doses of 2 mg histamine (6 mg histamine phosphate) dissolved in saline were employed. This drug had an augmentor action on all the strips, which resembled the records obtained with adrenaline: a marked and rapid shortening of the muscle and some increase in amplitude of rhythmic movements were recorded. The effect passed off rapidly, the tissue resuming its previous length in approximately 10-15 minutes. During the augmentor stage, the action of adrenaline was reversed, addition of the drug producing a relaxation. The inhibition was not always followed by recovery, since the effect of histamine passes off during the inhibition. The reversal was only elicited from the longitudinal fibres of the upper body and fundus of the rabbit's stomach.

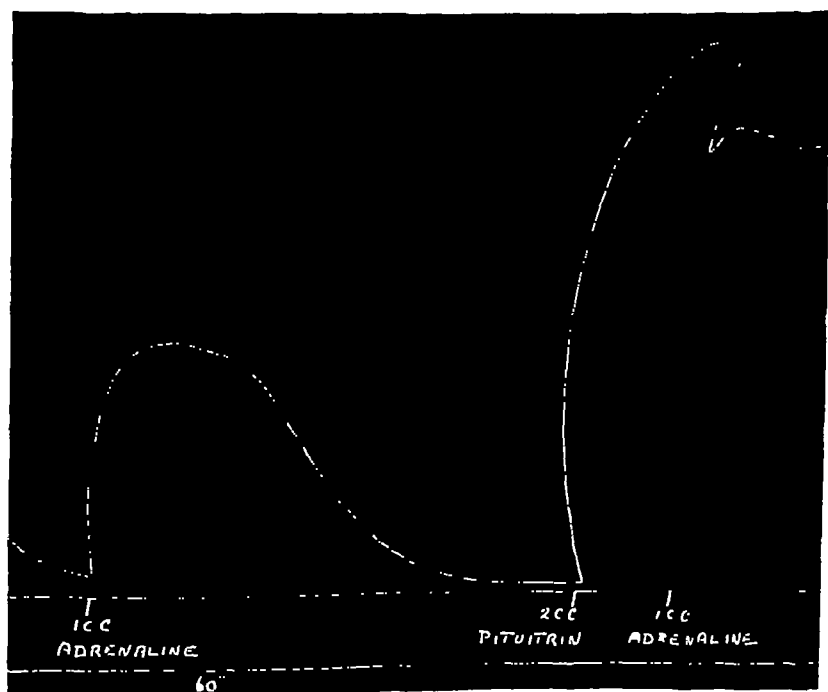


Fig 1 Fundus of rabbit's stomach longitudinal strip records showing reversal of response to adrenaline after addition of pituitrin

(c) *Pituitrin* The action of pituitrin closely resembled that of histamine, but in contradistinction to histamine, rhythmic contractions were usually diminished. The augmentor effect passed off gradually, and the strips resumed their previous length in some 20 minutes. Reversal of the adrenaline action was recorded in the longitudinal fibres of the upper body and fundus, but not in the other regions. In many experiments, incomplete reversal was produced, a diminished augmentor effect being preceded by a rapid inhibition.

(d) *Barium chloride* This drug caused in the preparations used a slow but sustained contraction of the muscle. In the majority of our experiments, the contraction was well maintained, the lever showing no tendency to return to the base line. Addition of adrenaline which previously caused an augmentor effect, produced after the addition of barium chloride a well-marked inhibitor response. The reversal effects obtained after the use of either pituitrin or barium chloride are of interest, as these drugs are described as acting directly on the muscle fibres.

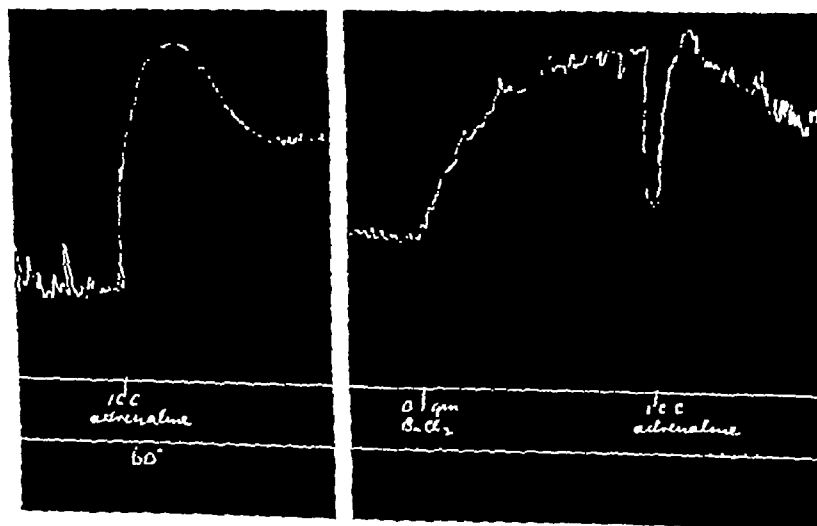


Fig 2 Fundus of rabbit's stomach, longitudinal strip reversal of response to adrenaline after addition of barium chloride

If, as in many experiments, the contraction produced by pituitrin and histamine was less than that obtained with the first dose of adrenaline, then the second dose of adrenaline seldom caused a definite



inhibitor response, the record showing at the most a slight preliminary inhibition

(e) *Atropine* By the use of atropine, further evidence may be advanced to show the relation between the length of the muscle fibre and the response to adrenaline. If the shortening of the muscle fibres predisposes to an inhibitor response, lengthening of the muscle by atropine might be expected to bring about a return of the augmentor response. Atropine was added to strips in which reversal had been produced by either pilocarpine, histamine or pituitrin, and if the relaxation recorded was sufficiently great, addition of adrenaline then caused an augmentor effect. Thus it was possible to obtain in the one strip with equal doses of adrenaline, an augmentor action, then an inhibitor action, and finally, an augmentor action.

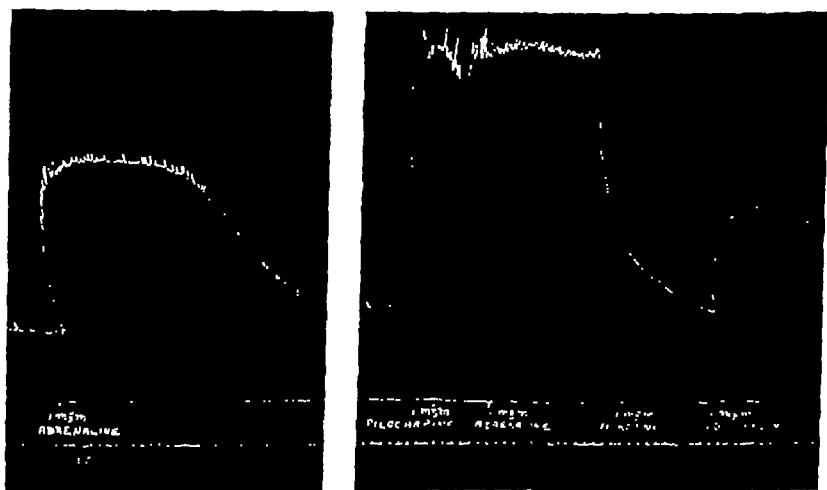


Fig 3 Fundus of rabbit's stomach, longitudinal strip, reversal of response to adrenaline after contraction with pilocarpine, recovery of augmentor effect of adrenaline after relaxation with atropine

We found the augmentor reaction of a strip to adrenaline to be in no way impaired by a dose of atropine sufficient to neutralise the action of subsequent doses of as much as 4 mg of pilocarpine.

If adrenaline be considered as acting on a motor and inhibitor mechanism of the neuro-muscular apparatus, then the reversal effects obtained could be accounted for on the assumption that the motor mechanism is thrown out of action either by the physical changes

accompanying the shortening of the muscle fibres or by the production of some substance in the muscle by the stimulating drugs. There is, further, the possibility, suggested by Langley (7), that reversal action may be due to the displacement of one absorbed substance by another. The ability, however, of adrenaline to reverse its own action would appear to disprove the latter suggestion.

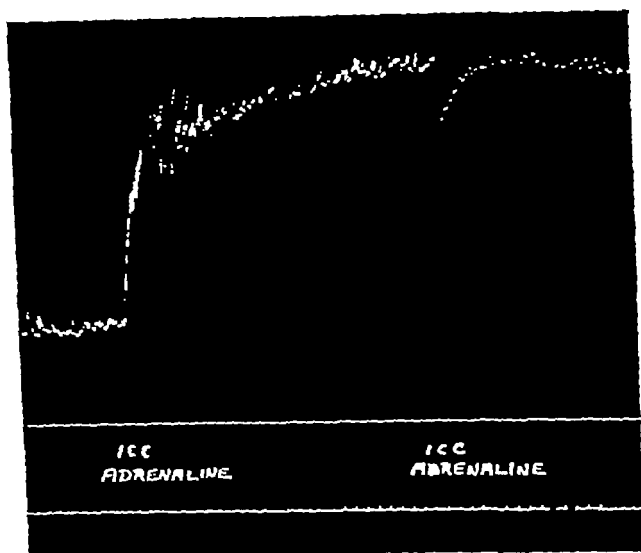


Fig 4. Fundus of rabbit's stomach, longitudinal strip, inhibitor effect of adrenaline when given during contraction produced by previous dose

Thus adrenaline was added to strips from the longitudinal fibres of the fundus, and during the contraction produced, a second dose of the drug was given. This was followed in many experiments by a typical inhibitory response such as is obtained after the use of stimulating drugs.

*Ergotamine* was employed in our later experiments, as this drug, or *ergotoxine*, as shown by Dale (8), has the property of paralysing the motor endings of the sympathetic. After a motor response had been obtained with adrenaline, 10 mg of *ergotamine* (Sandoz) were added. The addition of the drug caused no change in the activity of the preparation, but on addition of adrenaline, a marked inhibitory response was recorded which persisted even after a relaxation had been obtained with *atropine*. In the regions of the lower body, where rhythmic activity was well seen with but little or no tonus change, adrenaline after ergo-

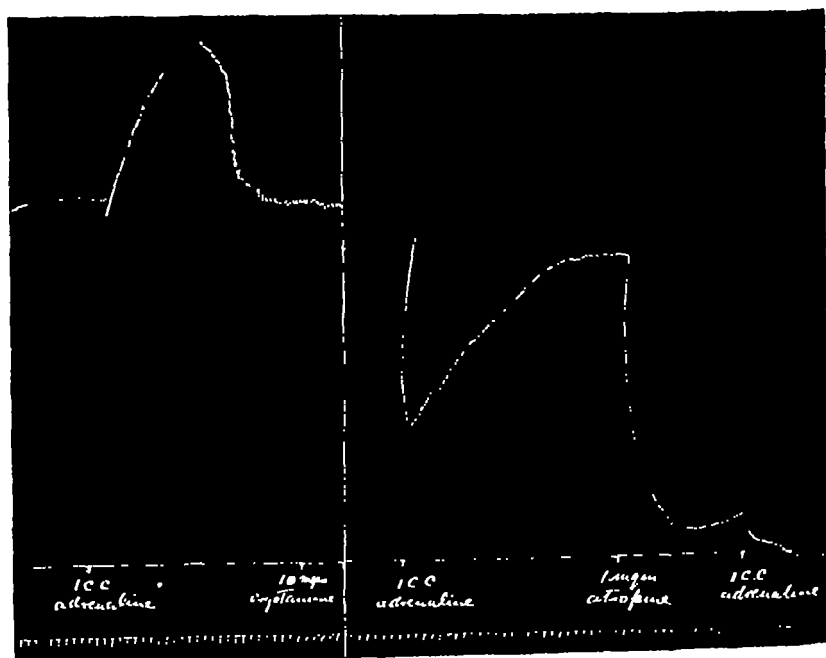


Fig 5 Fundus of rabbit's stomach, longitudinal strip reversal of action of adrenaline by ergotamine, persistence of inhibitor response after relaxation with atropine

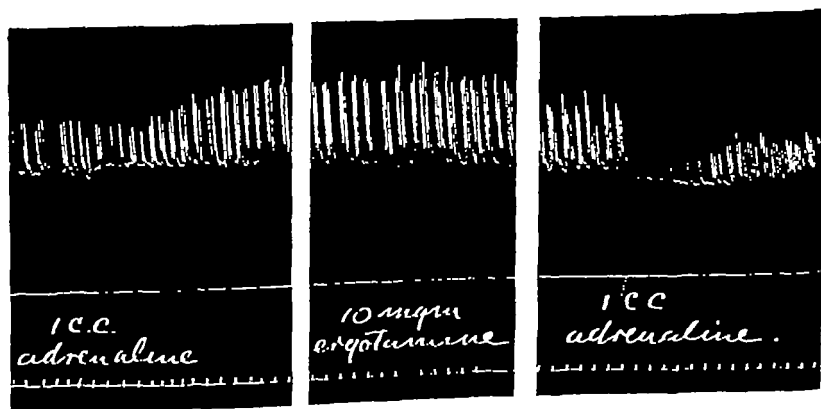


Fig 6 Lower body of rabbit's stomach reversal of action of adrenaline by ergotamine, augmentor and inhibitor effects of adrenaline involving rhythmic contractions only

tamine produced a marked decrease in the frequency of contraction, but no change in base line

*Fundus of the dog's stomach* In our previous experiments on the fundus of the dog's stomach, an inhibitor response was always obtained with adrenaline, though Smith<sup>(9)</sup> and Kuroda<sup>(10)</sup> recorded an augmentor effect. We have recently been able to obtain a similar effect by keeping the preparation for some hours at 0° C before suspending it in the bath at a temperature of 38° C. An inhibitor effect was observed with adrenaline when the muscle was tonically contracted.

*Guinea-pig's uterus* We have repeated the experiments described by Cow on the isolated uterus of the guinea-pig and have obtained an augmentor response with adrenaline instead of the usual inhibitor effect, by leaving the preparation in Ringer-Locke solution containing 1 in 5000 pituitrin for 1-2 hours at room temperature. The presence of pituitrin is not essential as the same augmentor effect is obtained on leaving the tissue in cold Ringer-Locke solution alone. The experiments were performed with strips of the cornu. Oxygen was bubbled into the solution as in our previous experiments the temperature of the bath being kept constant at 38° C.

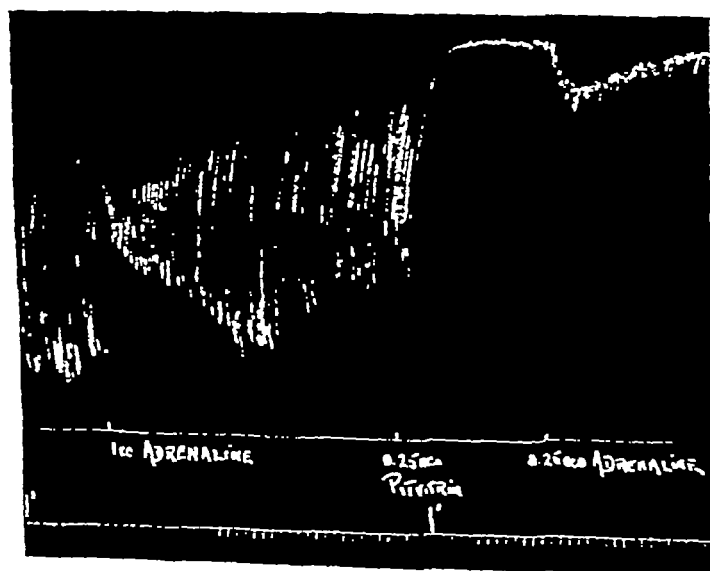


Fig 7 Guinea pig's uterus non pregnant left in Ringer Locke solution, containing 1/5000 pituitrin for 1 hour at room temperature augmentor response to adrenaline reversed after contraction with pituitrin.

Reversal of the effects of adrenaline was obtained in these experiments where a preliminary response had been recorded with adrenaline. The addition of 0.1 c.c. of pituitrin caused a marked shortening of the muscle fibres with some augmentation of rhythmic movement. The addition of adrenaline now elicited, in the great majority of experiments, a relaxation of the muscle fibres.

The action of histamine in doses of 1 mg. closely resembled that of pituitrin. The effect of adrenaline on the muscle was reversed, a well marked inhibitor response being recorded.

*Rabbit's uterus* Preparations were made of the cornu of the uterus of the rabbit, circular and longitudinal strips being used. Addition of adrenaline caused the usual augmentor effect, the tracing showing a marked increase of tone and augmentation of movement. Pituitrin in doses of 0.1 c.c. produced a marked rise of the lever, the tissue remaining in a state of sustained contraction for some 4-5 minutes before rhythmic movements returned with gradually increasing amplitude. A reversed response to adrenaline was never obtained, further addition of the drug causing either a continuation of the tonic contraction or a further

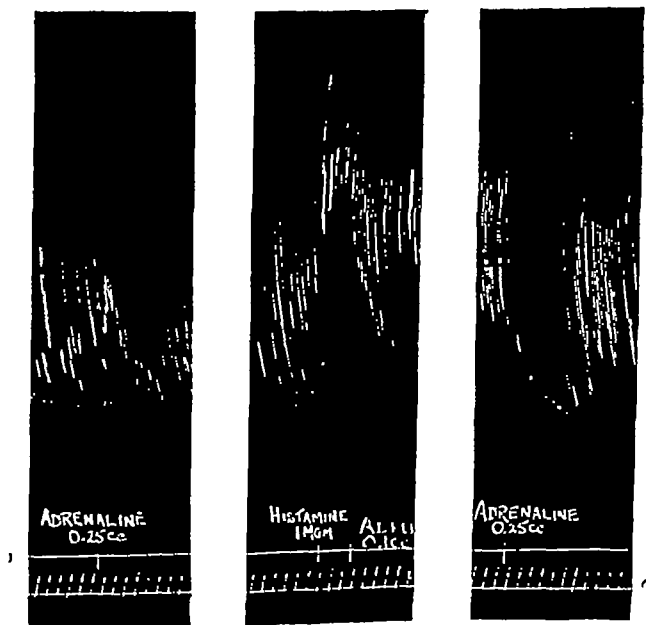


Fig 8 Rabbit's uterus, non pregnant, reversal of response to adrenaline after contraction with histamine.

increase in tone Histamine added in doses of 1 mg had a similar action to pituitrin, but the reaction to adrenaline was reversed, the drug causing inhibition of rhythmic contractions and in most experiments a marked fall in tone

*Ergotamine* The addition of ergotamine in doses of 5 mg caused no appreciable change in tone or rate of rhythmic contractions in either the rabbit or guinea-pig's uterus, with the subsequent addition of adrenaline, a relaxation of the muscle fibres and/or an inhibition of rhythmic movements The type of tracing depended on the existing condition of tonus

*Oxygen supply* We have performed experiments to demonstrate the action of adrenaline on the various preparations after the oxygen supply to the bath had been cut off The fundus of the rabbit's stomach relaxed on cessation of bubbling and addition of adrenaline invariably caused an increase of tone Strips of the cornu of the rabbit's uterus relaxed if tone was high and rhythmic movements were usually inhibited if tone was low, rhythmic movements only were affected The action of adrenaline under these conditions was always augmentor With preparations of the guinea-pig's uterus, cessation of bubbling caused a rise in tone and the muscle fibres became tonically contracted Adrenaline now caused an inhibitor effect, a reversal of the action of the drug, as in preliminary treatment of the strip, an augmentor effect was obtained

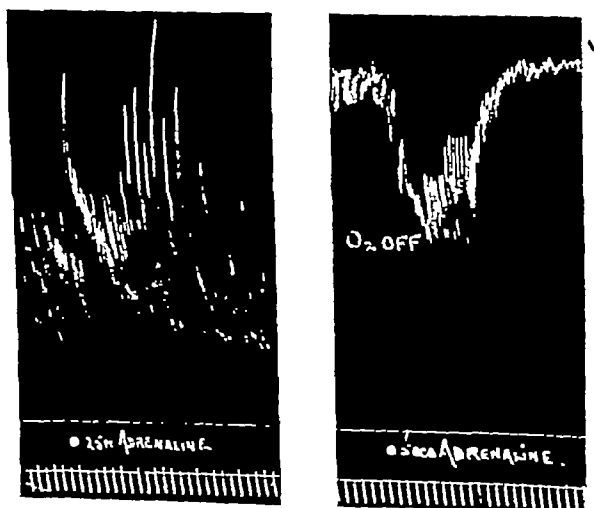


Fig 9 Guinea pig's uterus, pregnant augmentor action of adrenaline reversal of adrenaline action after muscle contracted on cessation of oxygen supply

From these experiments, lack of oxygen in itself would appear to have no connection with the response obtained with adrenaline

### DISCUSSION

A relationship clearly exists between the condition of the tissue and the nature of the response elicited by adrenaline, an inhibitor effect being obtained with the contracted or tonic muscle, and an augmentor response from the long or atonic strip. As these effects are obtained after the use of diverse drugs, some of which are regarded as acting on the parasympathetic system, others directly on the muscle, it would appear probable that the reversal effects recorded are due to changes accompanying variations in the condition of the muscle substance.

By the use of ergotamine, the inhibitor response to adrenaline may be obtained in the preparations described, which previously gave an augmentor response. The presence of an inhibitor mechanism demonstrated by ergotamine does not necessarily imply that reversal of the action of adrenaline can be produced merely by shortening the muscle fibres by drugs. In certain preparations which normally give a motor response to adrenaline, namely, the retractor penis of the dog and the cardia of the cat, we have been unable to obtain results similar to those described for the fundus of the rabbit's stomach. Edmunds<sup>(11)</sup> has, however, obtained relaxation of the retractor penis after addition of ergotamine, and we have recorded similar results with preparations of the cat's cardia. It is possible that, under certain conditions, relaxation may be obtained with adrenaline after shortening of the muscle fibres by other means, just as in experiments on the rabbit's uterus, an inhibitor effect was only observed after the use of histamine. In the region of the lower body of the rabbit's stomach, an inhibitor effect can only be obtained after ergotamine.

Accumulation of metabolites or any other results due to cessation of oxygen bubbling had no specific effect on the reaction of the tissue to adrenaline. With tissues which relaxed on stopping the oxygen supply, augmentor response was recorded from the relaxed muscle, while on the other hand, with preparations that contracted on cessation of oxygen bubbling, an inhibitor response with adrenaline was obtained from the contracted strip.

In view of the recent suggestion by Burn and Dale<sup>(12)</sup> that the relaxation of the capillaries obtained with a second injection of adrenaline is due to the production of histamine or some such substance in the body, it is interesting to note that we have obtained relaxation of the

rabbit's fundus with a second dose of adrenaline when the muscle remained tonically contracted after the first dose

The reversal of the normal response of strips of the dog's stomach and the uterus of the guinea-pig on keeping the preparation in cold Ringer or Tyrode's solution is of interest, as Cow only obtained the augmentor response of the guinea-pig's uterus with adrenaline after "sensitising" the preparation with pituitrin. He argued that there is normally an interaction of the secretion of the pituitary and suprarenal glands in the body during pregnancy.

Our experiments suggest that the mechanism controlling the augmentor and inhibitor response to adrenaline may be present in all preparations of smooth muscle capable of active contraction and relaxation.

#### SUMMARY

1 In certain regions of the rabbit's stomach, adrenaline elicits an augmentor response.

2 Strips of the fundus of the dog's stomach and the guinea-pig's uterus are contracted by adrenaline if the preparations are left in cold Ringer-Locke solution for 2-3 hours.

3 If the muscle fibres of the rabbit's fundus are shortened by pilocarpine, choline, histamine, pituitrin or barium chloride, the rabbit's uterus by histamine, the guinea-pig's uterus by pituitrin or histamine, an inhibitor response is obtained with adrenaline.

4 An inhibitor response may be changed into an augmentor response by certain agencies causing a lengthening of the muscle fibres.

5 Ergotamine, which in the doses used does not cause any change in the length of the muscle fibres, brings about a reversal of the action of adrenaline.

6 The type of response produced by adrenaline is related to the condition of tonus of the gastric musculature of the rabbit, an inhibitor response being obtained in the tonic or contracted muscle, an augmentor response in the atonic or relaxed muscle.

The expenses of this investigation were defrayed by a grant from the Government Grants Committee of the Royal Society, to whom our best thanks are due.



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# THE BUFFERING POWER OF THE BLOOD OF *MAIA SQUINADO* BY PHYLLIS M TOOKEY KERRIDGE<sup>1</sup>

(From the Department of Physiology and Biochemistry,  
University College, London )

THE purpose of this research was to investigate the buffering power of the blood of a marine animal with a view to comparison with that of mammalian blood. *Maia squinado* was chosen as a suitable subject for the experiments, as it could be obtained easily, and in large quantity, at the Marine Biological Laboratory at Plymouth, the yield of blood per individual is fairly large, and the blood does not coagulate.

Buffer curves have been obtained for both oxidised and reduced *Maia* blood, the reaction being varied by exposing the blood to gas mixtures containing varying proportions of carbon dioxide.

Very little work has been done previously on the subject. Quagliariello<sup>(1)</sup> measured the pH of *Maia* blood, using a hydrogen electrode, and found it to be 7.45. He also found that 5 mg equivalents NaOH added to 1 litre of the blood changed its pH to 9.0, and 10 mg equivalents HCl similarly to 4.0. Parsons and Parsons<sup>(2)</sup> determined the CO<sub>2</sub> content of *Maia* blood at varying tensions of CO<sub>2</sub>, and calculated the pH of the blood from the Henderson-Hasselbalch equation.

*Experimental methods.* The blood was collected from the specimens for these experiments by cutting off the legs of the animal, and collecting the fluid which dripped from the broken ends. Large quantities of blood were not required, and by this method it was easy to obtain a sufficient amount uncontaminated.

In order to eliminate individual variations, the blood from four or six animals was mixed before sampling. The clear blood was separated from the leucocytes and a small amount of sodium fluoride was added to prevent formation of fixed acid, and also thymol as a preservative. The experiments were always made on the same day as that on which the blood was collected.

Improvised tonometers were made from 250 cc round-bottomed flasks, fitted with corks with one hole, through which a short glass tube

<sup>1</sup> Working for the Medical Research Council.

was pushed, the end of which was flush with the inside surface of the cork. The top of the cork was covered with Everton's vacuum wax to prevent leakage when the flask was evacuated. A short length of rubber pressure tubing wired on to the glass tube enabled the "tonometer" to be attached to the gas-filling apparatus. A good screw clip on the tubing functioned instead of a tap. The flask could be emptied by inserting a small glass jet in the open end of the rubber tubing, inverting the flask and unscrewing the clip. The outward flow of liquid was facilitated by placing one's hand over the bulb of the flask, and so warming slightly the gases inside, which thus expelled the liquid.

The apparatus for filling the flask with gases of known composition was essentially a glass tube with five side arms, each fitted with a good tap, the flask to be filled being joined on to the end of the tube by rubber tubing. To one arm was attached a mercury manometer, one arm led to a water pump, and the other three to the supplies of hydrogen, carbon dioxide, and air respectively. The hydrogen was prepared from zinc and hydrochloric acid and was purified by passing through wash bottles of water, sodium hydrosulphite and caustic soda. The carbon dioxide was prepared in a Kipp apparatus from marble and hydrochloric acid, and was washed by bubbling through two wash bottles of water.

It was arranged that the carbon dioxide inlet was nearer the flask than either the hydrogen or air supplies, so that when working with small tensions of  $\text{CO}_2$ , this gas could be washed into the flask by the hydrogen or air admitted subsequently. Otherwise, the diffusion and mixing of the gases in the narrow bore of the filter being slow, inaccurate results were obtained. The mercury manometer was read to  $\frac{1}{2}$  mm Hg by means of a magnifying glass.

Two c.c. of blood were pipetted into each of six flasks, which were then in turn evacuated, and refilled with the requisite gas mixture. The blood was then equilibrated by violent agitation of the flasks in an eccentric bottle shaker. Three-quarters of an hour was found to be sufficient time for equilibration at the lower  $\text{CO}_2$  tensions, but at the higher tensions, *e.g.* 50 mm and over, two or three hours were essential.

The hydrogen ion concentration of the solution was determined by the glass electrode method (8,9). The blood was run straight from the equilibrating flask into the electrode, the first few drops being rejected, and the sample was immediately covered with liquid paraffin. A portable outfit incorporating a Lindemann electrometer was used.

*Results* A buffer curve of oxidised and reduced *Maria* blood is shown

in Fig 1, which is drawn from the results of five experiments on a total of 28 specimens of *Maia*. The figures are given in Table I. The pH

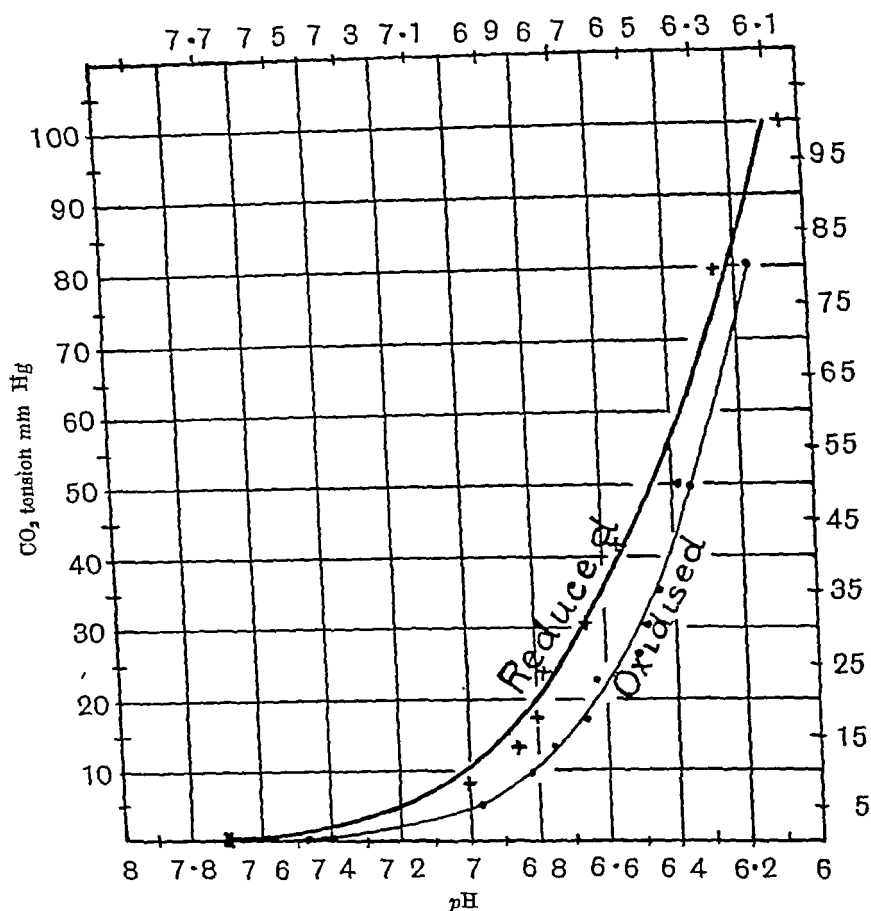


Fig 1.

determinations and the equilibrations were all done at room temperature, in rooms with a north aspect. The average temperature during the series of experiments was  $13^{\circ}\text{C}$ , the variation not being greater than  $\pm 1^{\circ}\text{C}$ . The error in the pH measurements was of the order  $\pm 0.1$  pH.

#### DISCUSSION

It is obvious from the results obtained that *Maia* has a considerable buffering action towards  $\text{CO}_2$ . This is greater than that of sea water,

TABLE I.

	CO <sub>2</sub> tension mm Hg	pH
(a) Oxidised blood	0 0	7 03
	5 75	6 96
	10 0	6 82
	13 0	6 92
	18 0	6 64
	22 5	6 62
	27 0	6 50
	30 5	6 49
	36 0	6 43
	50 0	6 34
	50 0	6 39
	81 2	6 16
(b) Reduced blood	0 0	7 71
	8 5	7 01
	13 0	6 87
	17 5	6 80
	24 5	6 77
	31 5	6 64
	41 3	6 55
	79 5	6 25
	100 75	6 04

but not as great as that observed in mammalian blood especially at low tensions of CO<sub>2</sub>. Fig 2 gives the change in cH with CO<sub>2</sub> tension, and

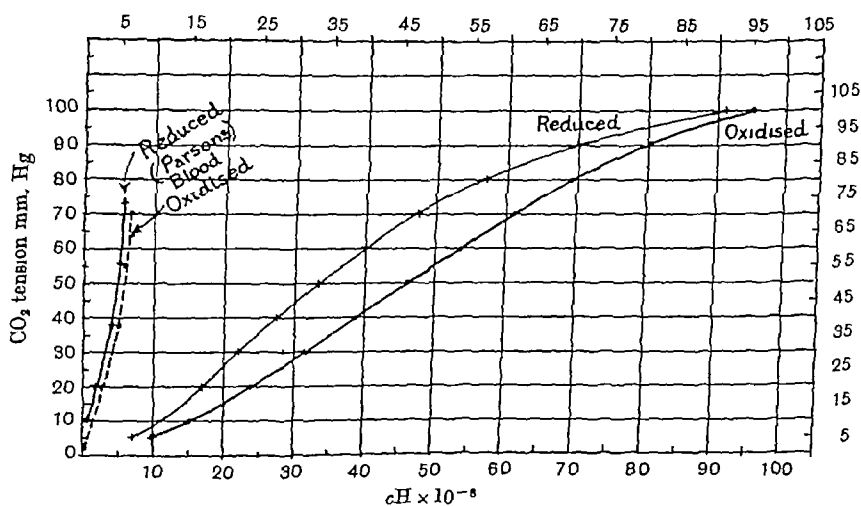


Fig 2

shows the relation to be approximately a straight line one between 10 and 70 mm CO<sub>2</sub>. The results of similar experiments, made by Parsons on his own blood, are included in the figure for the sake of comparison

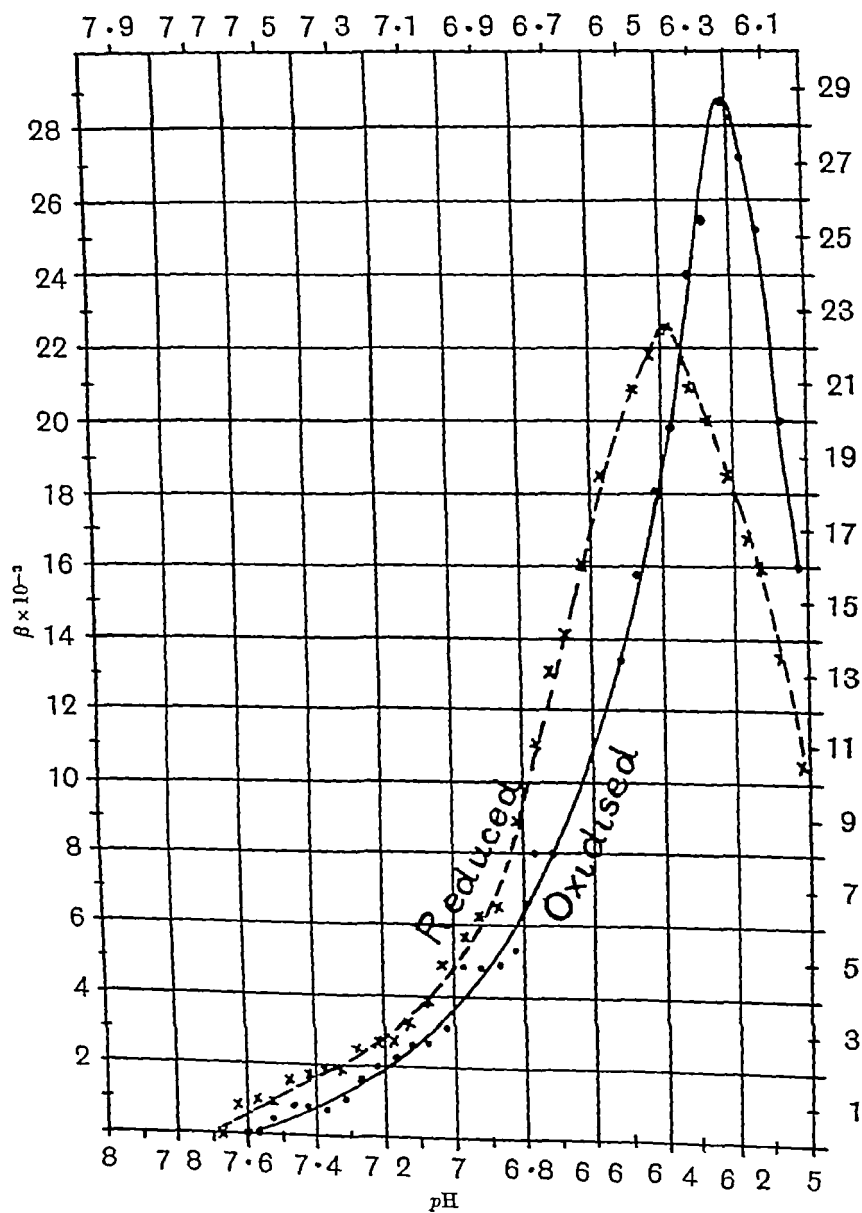


Fig 3

Fig 3 shows the variation of buffer power with  $pH$ . For the purpose of this figure, the definition of buffer power ( $\beta$ ) as given by v Slyke(3) has been adopted

$$\beta = \frac{dB \text{ (in millimols)}}{d pH},$$

where  $dB$  represents the amount of alkali or acid in millimolecular concentration which must be added to the solution in order to change its reaction by a small amount  $d pH$ . The curves have been obtained by taking the tangent to the curves in Fig 1 at intervals of 0.05  $pH$ . The buffering power rises to a maximum value at  $pH$  6.39 for reduced blood, and 6.205 for oxidised

Since the blood of *Mara* is homogeneous, it is probable that a Henderson-Hasselbalch equation expresses the equilibrium

$$pH = pK' + \log \frac{(\text{salt})}{(\text{acid})},$$

where (salt), (acid) have been taken to represent the total concentration of the salts and undissociated acids in the *Mara* blood which are acting as buffers towards the  $CO_2$  in this range of  $pH$ , and  $pK'$  being the negative logarithm of the mean dissociation constants of the acids, divided by the degree of dissociation of the salts under the existing circumstances of dilution. This equation follows directly from the Law of Mass Action of Guldberg and Waage applied to dilute solutions.

It can be shown(3) that the buffering power is at a maximum when  $pH = pK'$ , which occurs when the concentrations of buffer acids and their salts are equal. Having found the  $pH$  at which maximum buffering takes place by experiment, we may deduce

$$pK'_r = 6.39$$

$$pK'_o = 6.205$$

or

$$K'_r = 4.07 \times 10^{-7}$$

$$K'_o = 6.24 \times 10^{-7}$$

where the suffixes "r" and "o" serve to indicate the reduced and oxidised states respectively.

Assuming that the buffer salts are practically fully dissociated at the dilutions in blood (which is approximately true), we have thus figures for the mean dissociation constants of the acids in the blood which take part in the buffering. These are of the same order as the first dissociation constant of carbonic acid itself ( $3 \times 10^{-7}$ ). Reduced and oxidised haemocyanin, both being weak acids, play a part in the

buffering Oxyhæmoglobin, being apparently a stronger acid than the others present, has increased the mean dissociation constant  $K'$ .

Van Slyke has also shown that all monovalent acid buffers with values of  $pK'$  between 4 and 10 which follow the Henderson-Hasselbalch equation, have the same maximum molecular buffering power. The molecular buffering power ( $\beta_m$ ) is defined as the buffering power ( $\beta$ ) divided by the total molecular concentration of buffers ( $C$ )

$$\text{Thus} \quad \beta/C = \beta_m$$

and at maximum buffering,

$$\beta_m^{\max} = 0.575$$

hence

$$\begin{aligned} C &= \beta_{\max}/0.575 \\ &= 0.5 \text{ } N \text{ for oxidised Maia blood} \\ &= 0.4 \text{ } N \text{ for reduced Maia blood} \end{aligned}$$

Van Slyke's figure for the total buffer concentration in mammalian blood is 0.057  $N$ .

The carbon dioxide which is taken up by blood in the range of  $pH$  which we have been considering must exist in the blood mainly in the form of bicarbonate as the normal carbonate does not exist in appreciable quantities at this  $pH$ . A small amount will be in solution as  $H_2CO_3$ . The amount of combined  $CO_2$  can be calculated from the  $CO_2$  tension, the  $pH$  and the Henderson-Hasselbalch equation. The first dissociation constant of carbonic acid, as determined by Walker and Cormack<sup>(4)</sup> is  $3.0 \times 10^{-7}$  thus  $pK$  for a bicarbonate equilibrium is 6.5, assuming that  $NaHCO_3$  is fully dissociated at this dilution. The calculated amounts of bicarbonate in the Maia blood are shown in Fig. 4 together with the measurements of Parsons and Parsons<sup>(2)</sup>. The agreement between the two curves is very good up to 80 mm.  $CO_2$ . The greater capacity of reduced blood for carbon dioxide than of oxidised is well brought out in this figure. Oxyhæmoglobin being a comparatively strong acid, can combat with carbon dioxide for the available base. The decrease in the  $CO_2$  bound by both oxidised and reduced bloods at tensions of 70 mm. and over is probably due to some irreversible change which takes place in the blood at about  $pH$  6.0. The effect cannot be due to insufficient equilibration as increased acidity would exaggerate the effect. It is possible that the high figure of Parsons and Parsons at this tension may have been due to this cause. Hogben<sup>(5)</sup> in the course of work on the oxygen capacity of Maia blood, also found an anomalous result about  $pH$  6.0. The tension of oxygen necessary for



50 p c saturation decreased progressively as the pH decreased until 6.0, after which it increased. A discussion of possible theories to account for this is given in Hogben's paper

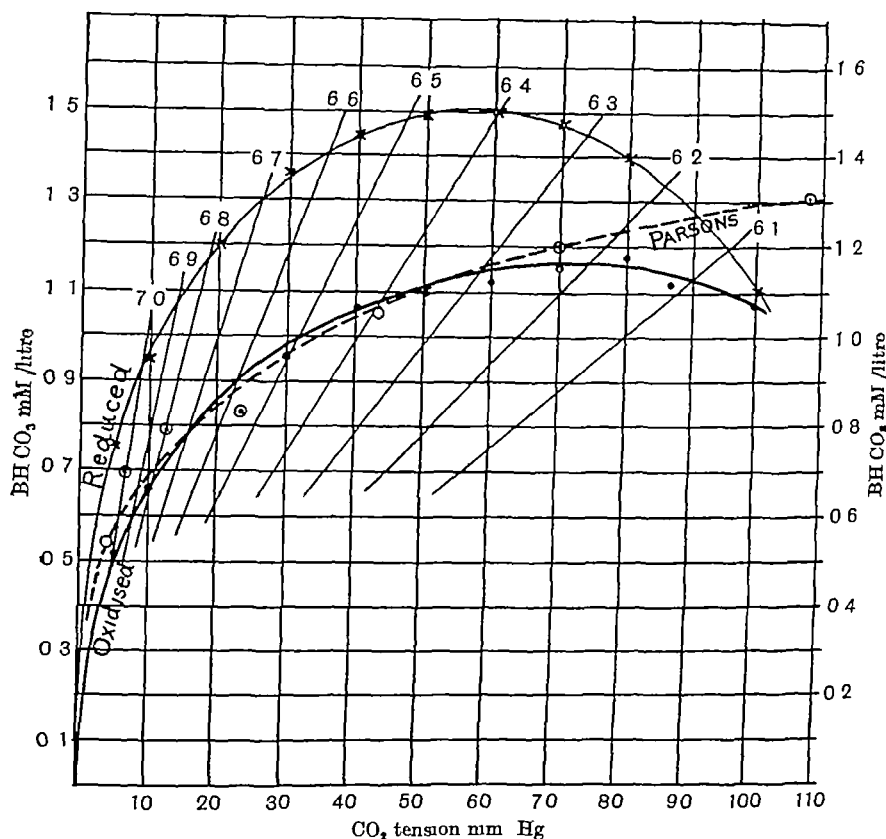


Fig 4.

The difference in the bicarbonate content of reduced and oxidised blood at constant pH is approximately 5 mM/litre over the range of pH 6.3-6.9. The oxygen capacity of *Mania* blood, as determined by Stedman and Stedman(6) is 1.14 vols p c or 51 mM/litre. Thus a combination with 5 mM of oxygen has rendered the blood unable to take up 5 mM of bicarbonate. If the assumption be made that this is due to oxyhæmocyanin possessing one or more acid groups which are dissociated to a degree comparable with that of the first H ion of carbonic acid, and to reduced hæmocyanin being little dissociated compared with the same standard, it follows that the number of dissociable

hydrogen ions in a molecule of oxyhæmocyanin is equal to the number of copper atoms in a molecule or aggregate Dheré and Schneider(7) have shown that the absorption of oxygen by hæmocyanin is directly proportioned to the copper content The S-shaped dissociation curve obtained by Stedman and Stedman suggests that the molecule or aggregate of hæmocyanin contains more than one atom of copper Until more work has been done on this point it is idle to speculate further

Finally, although the reactions occurring in blood containing hæmocyanin have been shown closely to resemble those in bloods containing hæmoglobin, yet it is doubtful whether *in vivo* the bicarbonate equilibrium plays such a large part in hæmocyanin bloods as in hæmoglobin bloods The conditions under which the bicarbonate content, or the buffering power, is great occur at tensions of  $\text{CO}_2$ , and at concentrations of hydrogen ion, which do not occur in the living animal.

#### SUMMARY

1 The hydrogen ion concentration of the blood of *Maia squinado*, oxidised and reduced, and exposed to various tensions of  $\text{CO}_2$  has been measured.

2 The buffering power of the blood, and its bicarbonate content under varying  $\text{CO}_2$  tensions have been calculated.

3 It has been shown that oxyhæmocyanin is a stronger acid than reduced hæmocyanin.

In conclusion, I wish to express my sincere thanks to Dr E J Allen, F.R.S., and the staff of the Marine Biological Laboratory, Plymouth, for their kind hospitality and help, and to Prof A. V Hill, F.R.S., for helpful criticism of the results obtained.

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# ON THE CELLULAR MECHANISM OF BILE SECRETION AND ITS RELATION TO THE GOLGI APPARATUS OF THE LIVER CELL

BY W CRAMER AND R J LUDFORD

THE secretion of bile by the liver cells presents an unusual problem of secretory cellular activity. In the cells of all other secreting glands the specific product, or products, of secretion are passed out of the cell in one direction only. In the pancreas, the salivary glands, and the gastric glands, the specific products of secretion are formed in the cells and passed into the ducts of the glands. In the kidney substances are removed from the blood and secreted into the tubules. In the internally secreting gland the specific secretions are passed into the blood. All these cells are thus functionally unipolar. But in the liver the cells are functionally bipolar. They form glycogen from carbohydrates, from proteins, and possibly also from fats taken up from the blood and secrete it as glucose into the blood stream. As one of us<sup>(1)</sup> has recently pointed out, all the known facts compel a return to the old conception of Claude Bernard that the glycogenic function represents an internal secretion of the liver cell. At the same time the liver cell takes up the bile pigments (which are now held to be formed by the cells of the reticulo-endothelial system<sup>(2)</sup>) and the bile salts from the blood and secretes them into the intercellular bile canaliculi. Each liver cell subserves, therefore, an internal and an external secretion. It is difficult to form a mental conception of the cellular mechanism which enables one cell to pass two different specific secretions in different directions. The following observations on the Golgi apparatus of the liver cell as demonstrated by the osmic acid impregnation method throw light on this problem.

Practically nothing is known concerning the Golgi apparatus in the liver cell, which is the more remarkable as in recent years a great deal of study has been devoted to this cytoplasmic structure in glandular cells. This may be taken as an indication of the difficulty of demonstrating it in the liver cell. The difficulty seems to be due mainly to the reason that the presence of glycogen in the liver cell is a disturbing factor. For in the process of fixation by a watery fluid the glycogen is washed out of the cells and thus probably distorts the morphological appearance of the Golgi apparatus. That, at any rate, is the most obvious

explanation of the irregular and broken appearance of the Golgi apparatus in liver cells containing a considerable amount of glycogen. In order to avoid this difficulty we have rendered the liver cells free, or almost free, from glycogen by various devices such as feeding with thyroid gland, injection of  $\beta$ -tetrahydronaphthylamin, exposure to cold, and injection of insulin. All these conditions produce a rapid diminution of the hepatic glycogen. It should be noted, however, that, as one of us<sup>(1)</sup> has shown, the disappearance of glycogen produced by insulin is due to an inhibition of the glycogenic function involving an inhibition of the formation of glycogen. The other conditions mentioned above act in exactly the opposite way. They stimulate the glycogenic function of the liver cells to increased activity so that glycogen is secreted into the blood stream in the form of glucose as rapidly as it is being formed. It is not known at present how the glycogenic function of the liver cell is related to its bile secreting function, so that we are not entitled to assume that the conclusions concerning the inhibitory action of insulin and the stimulating action of thyroid feeding and  $\beta$ -tetrahydronaphthylamin on the glycogenic function can be transferred to the bile secreting function. An increased formation and secretion of bile as the result of thyroid feeding has been demonstrated quite recently by Speidel<sup>(3)</sup> for tadpoles.

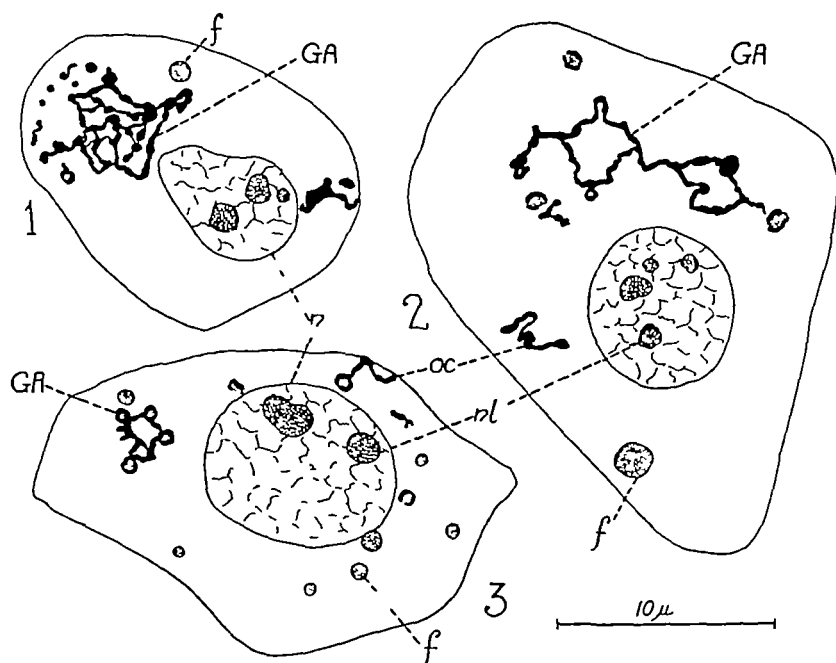
We have examined the livers of normal and pregnant mice, rats and guinea-pigs, and also the livers of the same animals following

- (a) Injections of insulin, pituitrin, and  $\beta$ -tetrahydronaphthylamin,
- (b) Feeding with thyroid and a tryptophane-free diet,
- (c) Exposure to cold,
- (d) Removal, after ligation, of a piece of the common bile duct

In every case the animals were killed with coal gas before removing small portions of their livers for fixation.

On examining preparations of the liver-cells for the Golgi apparatus by the modified Mann-Kopsch method (impregnation with osmic acid) one is at once struck by the great variety in its appearance in different cells. The form of the Golgi apparatus varies considerably in any one section of the liver, the variations being particularly well marked in the livers of pregnant animals. Figs 1-3 represent liver cells from a case of early pregnancy in the mouse. In Fig 1 the Golgi apparatus is in the form of a juxta-nuclear network, in Fig 2 the network is drawn out around the nucleus, while in Fig 3 there is a small network at one side of the nucleus and scattered osmophil fragments which may well have arisen from disintegration of such a network as that of Fig 2.

Particularly striking is the appearance presented by the liver cells following the injection of  $\beta$ -tetrahydronaphthylamin Fig 4 shows a



Figs 1-3 Liver cells from a mouse at an early stage of pregnancy  
GA, Golgi apparatus, f, fat, oc, osmophil canaliculi, n, nucleus, nl, nucleolus

group of cells from the liver of a guinea-pig following such an injection. There is no juxta-nuclear network. These cells show instead a number of branched and coiled osmophil filaments, or canaliculi (oc) in contact with the walls of the intercellular bile canaliculi. This condition of the cells is even better shown following ligation of the bile duct. Fig 6 shows a part of the liver of a mouse, the bile duct of which had been tied 1 hour previous to fixing the liver. The appearance is similar to that shown in Fig 4, except that the osmophil filaments (oc) are more distended and are clearly seen to be canalicular in structure. Fig 5 shows a single cell from the same liver, after staining the mitochondria with acid fuchsin.

Following the injection of insulin many cells show the Golgi apparatus in the form of a compact juxta-nuclear network, but by far the greater number of cells contain scattered osmophil canaliculi. Figs 7, 8 and 9 are cells from the liver of the mouse after the injection of insulin. Fig 7

shows a binucleate cell with a reticulate Golgi apparatus (*GA*) at one side of the nucleus and a number of osmophil canaliculi scattered throughout

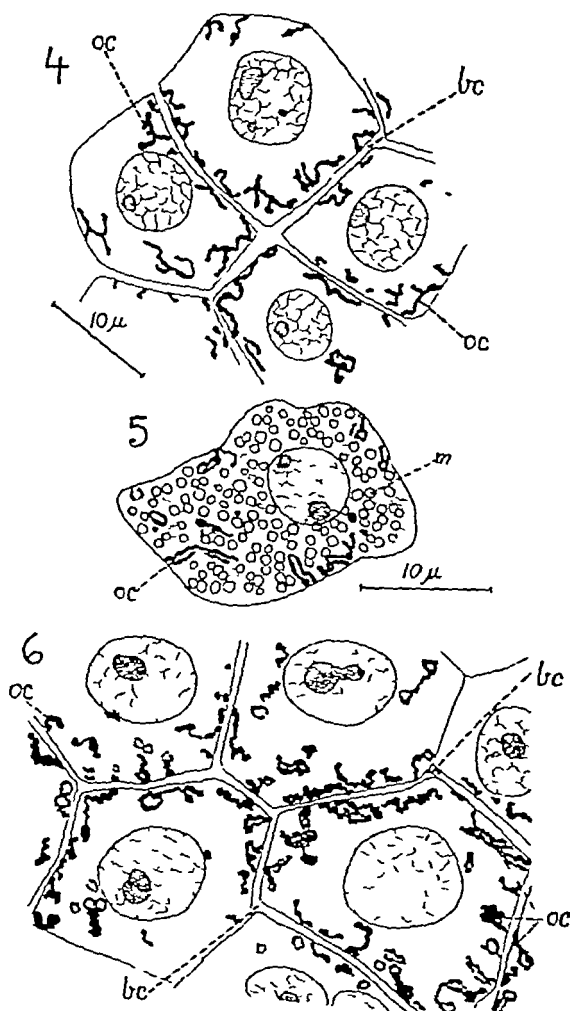
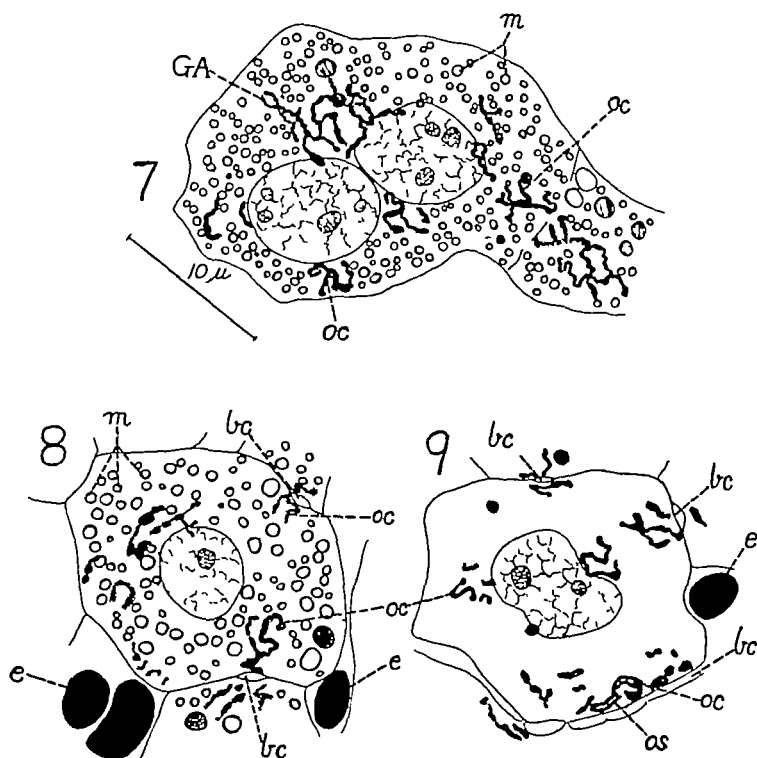


Fig 4. Liver cells of guinea pig previously injected with  $\beta$  tetrahydronaphthylamin.

Figs 5, 6 Cells from liver of mouse, the bile duct of which had been tied 1 hour previous to fixing the tissue Fig 5 with mitochondria stained with acid fuchsin.   
 oc, osmophil canaliculi bc intercellular bile capillaries, m, mitochondria.

the cytoplasm Such a condition is relatively rare, it being usual to find either a typical network or else scattered filaments Figs 8 and 9 are

more characteristic. It will be observed that in Fig 9 one of the canaliculi which is connected with the intercellular bile capillary (*bc*) can be seen

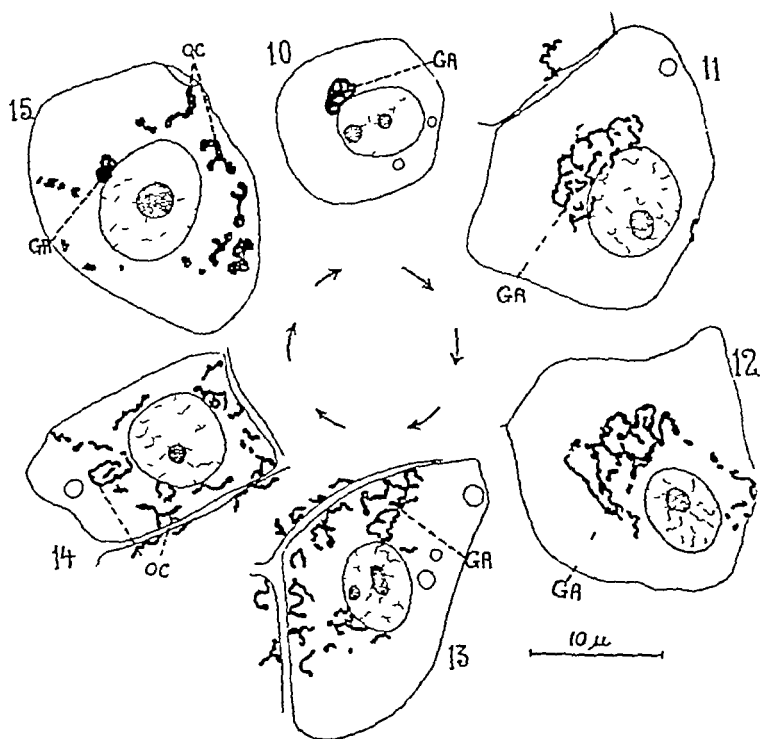


Figs. 7-9 Cells from liver of mouse which had received injections of insulin. *bc*, intercellular bile capillaries, *e*, red blood corpuscles *m*, mitochondria, *oc*, osmophil canaliculi, *os*, osmophil substance forming the wall of the intracellular canaliculi

to consist of a colourless interior surrounded by an osmophil substance (*os*)

There are thus two extreme conditions of the Golgi apparatus in the liver cells. One is represented by the small juxta-nuclear network (Fig 10) corresponding to the appearance of the resting Golgi apparatus in other gland cells. The other extreme is seen in Figs 4, 6 and 14, where osmophil canaliculi are seen lying at the periphery of the cell and making contact with the intercellular bile capillaries. The osmophil canaliculi in this latter condition resemble in appearance and arrangement so closely the intracellular bile canaliculi as figured by Kupffer and reproduced in Schäfer's *Essentials of Histology* that one cannot

but consider them as identical structures. Between these two extreme conditions which we have just described, various intermediate forms of



Figs. 10-15 General scheme showing the relation of the Golgi apparatus to bile secretion.

Fig 10 From rat fed on tryptophane free diet

Figs 11-14. From rats fed with thyroid gland.

Fig 15 From rat injected with pituitrin.

GA Golgi apparatus, oc, osmophil canaliculi.

the Golgi apparatus can be seen in different preparations. In some cells the Golgi apparatus is greatly enlarged and has moved away from the nucleus. In another preparation the Golgi apparatus appears in a fragmented form as numerous isolated threads scattered through the cytoplasm but not touching the periphery.

Recent investigations on the cytology of secretion have shown that in many externally secreting gland cells the specific secretion first appears as droplets within the osmophil material which constitutes the reticulum of the Golgi apparatus. This appears in the resting stage as a compact network applied to the surface of the nucleus, enlarges and spreads out



in the cytoplasm at the onset of functional activity, and finally it breaks up into fragments which are scattered through the cytoplasm and becomes reconstructed again later in the form of a compact network characteristic of the resting stage (4, 5, 6)

All these various phases of the Golgi apparatus can be seen in the liver cell and are illustrated by our drawings. As a pictographic summary of our observations drawings from different preparations have been arranged in Figs 10-15 so as to present the complete cycle as we conceive it to occur.

#### CONCLUSION

Our observations lead us to the conception that in the liver cells the bile constituents appear as a secretion within the Golgi apparatus, which in the process of bile secretion first enlarges and then fragments. The fragments are dispersed through the cytoplasm and reach the periphery where they pass their contents into the intercellular bile capillaries.

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# THE SENSITISING ACTION OF ALKALIES

By A HEMINGWAY

*(From the Department of Physiology, King's College, London )*

THAT the tone and activity of tissues containing plain muscle, such as blood vessels, intestine, uterus, etc., is largely dependent upon the acidity or the alkalinity of the fluids with which they are bathed has long been known. Amongst earlier papers those of Schwarz and Lemberger(1) and Hooker(2), working on blood vessels, showed the dilating power of acids. Lately, Evans and Underhill(3) have investigated in detail the changes in tone of plain muscle when subjected to fluids of various concentrations of hydrogen-ions. They showed that, within normal limits, increase of hydrogen-ion concentration caused relaxation of muscle, whilst a decrease had the opposite effect, increasing the tone and causing a quickening of the rhythmic movements.

In addition to these changes in tone and activity there is considerable evidence that certain drugs are more effective when used in an alkaline medium, and the present investigation has had its starting-point in this observation and more particularly in the work of McDowall(4) upon the relationship between the action of alkalies and pituitary extract upon pulmonary vessels. It is now shown that continuous exposure of the tissue to changes towards the alkaline side is not necessary for the exhibition of this action, but that sudden and repeated excursions towards slight alkalinity, with a return to normal following the change, are even more effective in bringing about the exaggerated response.

## *Method*

Three preparations have been studied, mammalian blood vessels, small intestine and uterus. In the first, the hind limbs of the cat have been perfused and changes in the peripheral resistance observed. This has been measured by using a scheme based upon that of Schafer(5). Fluid is fed from a Mariotte bottle (A) maintained at a suitable height. After passing through a heating coil (B), it is fed to a five-way tube (C) furnished with an air-trap, thermometer, connection to a side tube (D) and to the perfusion cannula. This cannula is inserted into the abdominal

aorta, and the resistance is measured in the side tube, and recorded by connecting to a piston recorder or a water manometer. All branches of

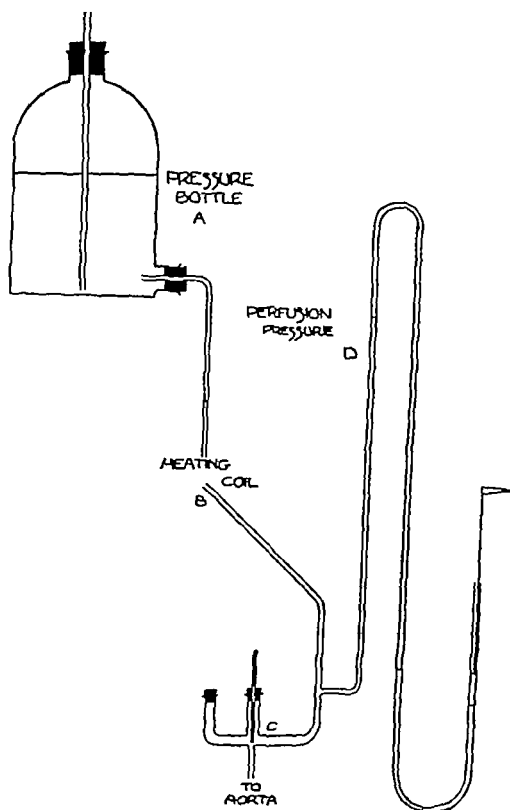


Fig 1 General arrangement of the perfusion scheme

the aorta to the intestine and pelvic viscera are ligatured and a return cannula inserted into the inferior vena cava

Any substances to be introduced into the perfusion stream were made up to be contained in 0.5–1.0 c.c. of solution. The needle of the syringe containing the fluid was pushed through the rubber tube leading to the aortic cannula and the addition made as rapidly as possible. This modification, suggested by Prof McDowall, ensures that an accurate record of the point of injection is taken since a small increase of pressure is caused momentarily in the manometer. In addition, there is no question as to the varying dilutions of successive injections which a slower rate of injection might introduce. Control injections of Ringer's

fluid show that no other effects are induced. In all the tracings shown, therefore, there is a small curve indicating the exact point of injection and this is then followed by a curve due to the reaction of the vessels, if any.

The perfusing fluid consisted of Ringer's solution ( $\text{NaCl}$  0.9 p.c.,  $\text{KCl}$  0.42 p.c.,  $\text{CaCl}_2$  0.24 p.c.), no buffer substances were added, and the solution was adjusted to  $\text{pH}$  7.4 by the addition of  $\text{N}/100$   $\text{NaOH}$  until it was slightly alkaline and finally brought back to the required concentration by blowing in  $\text{CO}_2$ .

The animals were killed by bleeding after preliminary anaesthetisation with chloroform and ether, and the procedure was then to commence the perfusion as speedily as possible (in some cases this being done whilst the circulation was still undisturbed and afterwards tying off the aorta and vena cava), but the rapidity with which the operation is performed does not appear to affect the results obtained. The perfusion pressure was then adjusted to the desired level and, a steady flow being established, injections of alkali were made into the stream of the perfusing fluid. Usually about 0.5–1.0 c.c. of fluid was added, the solutions used being  $\text{N}/100$   $\text{NaOH}$ , 5 p.c.  $\text{NaHCO}_3$  ( $\text{pH}$  8.5), and buffered phosphate solutions of  $\text{pH}$  9.

### Observations

The invariable response of the vessels to a change in hydrogen-ion concentration thus induced was a slight constriction, showing itself as an increase in the peripheral resistance, although sometimes this was almost imperceptible at the commencement. If, now, the injections were continued at, say, intervals of 2 minutes, the reaction of the vessels became gradually more pronounced until finally, after perhaps some 12

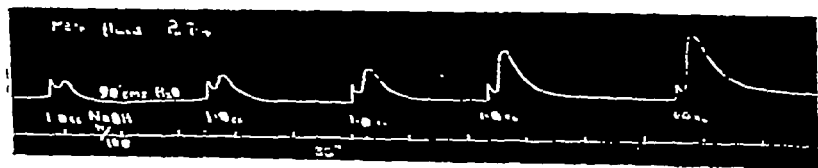


Fig 2 Perfusion of hind limbs of cat. Gradual increase of response to addition of alkali. The preliminary rise in each curve is due to the injection and this is followed by the rise due to vaso constriction.

injections, it remained fairly constant. The course of such an experiment is shown in Fig 2.

An attempt was made to determine what changes in alkalinity were

occurring during the reaction and in this particular experiment the perfusing fluid was at  $pH\ 7.4$ . The addition of 1 c c  $N/100\ NaOH$  raised the  $pH$  of the fluid issuing from the inferior vena cava to 7.5 within 30 seconds and 1 minute after the injection this had returned to normal again. So that the vessels were subjected to their original solution before the next addition of alkali was made.

Parallel to this increased sensitivity to alkali there runs an exaggerated response to adrenalin and also to pituitary extract. An early effect of the drug, before sensitisation has commenced, is taken as standard and

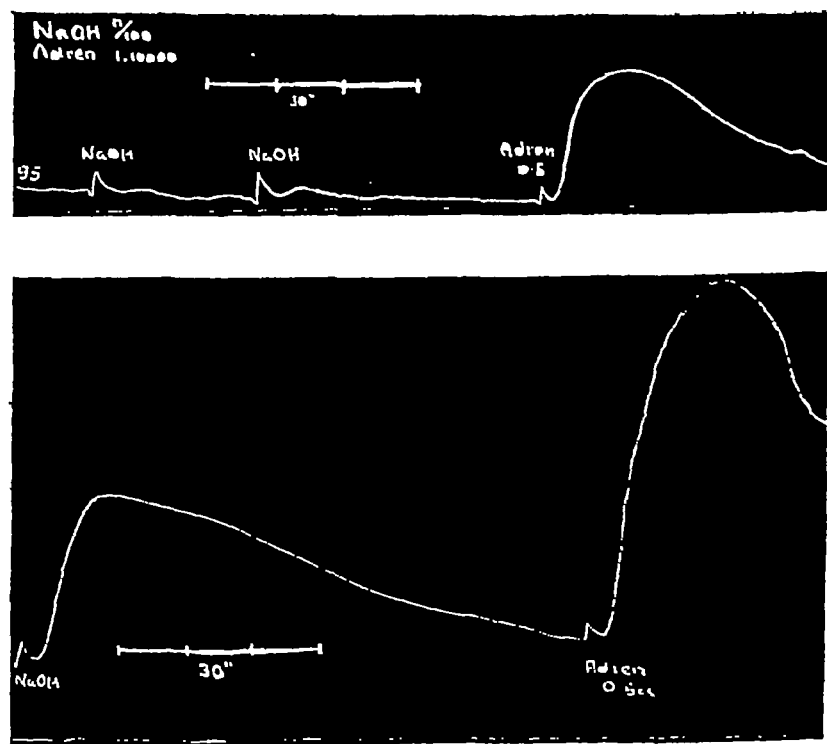


Fig 3 Sensitisation to adrenalin. The upper tracing shows the commencement of the perfusion with slight increase in response to the second addition of alkali. The lower tracing gives the reaction to the fourteenth injection of  $NaOH$  and the exaggerated response to adrenalin. Perfusion pressure in cm  $H_2O$ .

then, later, a second response is always much increased. A typical result is shown in Fig 3.

In the preliminary experiments either sodium hydrate or sodium

bicarbonate was used as the alkaline solution, and it was thought that the Na-ion might perhaps play some part in bringing about the results. To test this possibility buffered solutions of sodium phosphates and potassium phosphates were prepared of the same alkalinity, and, as expected, could be interchanged without altering the degree of response. The changes, therefore, are brought about by differences in hydrogen-ion concentration.

This confirms the result of Schmidt(6), who pointed out the importance of hydroxyl-ions in determining the response of vessels to adrenalin.

If the increased excitability is associated with the decrease in the hydrogen-ion concentration, then additions of acids to the perfusing fluid should bring about a desensitisation. To verify this idea a preparation was first rendered sensitive in the usual way and then this was shown to be reduced by repeated injections of HCl, for after three or four injections of acid and the reaction of the returning perfusing fluid having returned to the original state, the constriction due to the alkali was considerably reduced, but could be increased again by further alkali, the reaction being reversible (see Fig 4)

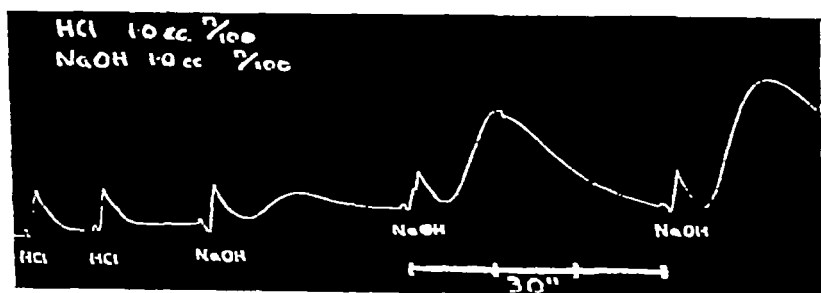


Fig 4. Showing resensitisation after disappearance of the effect with injections of acid.

The uterus and the small intestine of the cat were found to give similar results, in addition to observations made upon the pulmonary vessels. The changes in alkalinity in the case of the former tissues were brought about by rapidly filling the vessel containing them with a solution of sodium bicarbonate pH 8.5 or adding 1 cc alkali to the solution, immediately emptying and washing two or three times with Ringer's solution. With the uterus the response to histamine and pituitary extract was determined before and after treatment with alkali, and for the intestine, pilocarpine was used as the stimulating agent. In both cases

an increased response was elicited after washing with alkali, although the time of exposure to the changed solution was not more than 20 seconds (see Fig 5) The response showed a slight increase with each

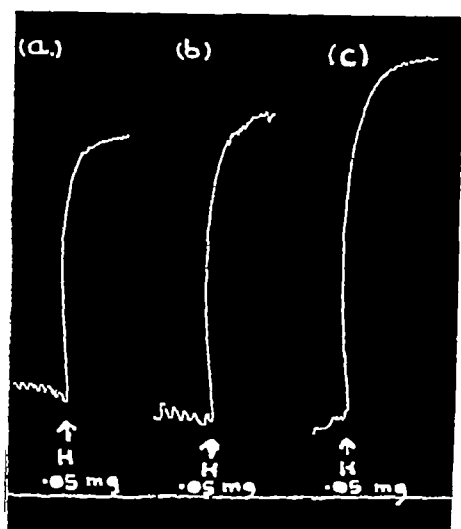


Fig 5 Increasing contractions of the uterus to a constant amount of histamine added to 100 c c. of Ringer's solution. After each addition, the tissue was bathed in  $\text{NaHCO}_3$  solution pH 8.5 and then washed

washing for three or four times and then became constant, or changes in the general tone of the preparation made further accurate comparison impossible

In conclusion, it may be said that the response of plain muscle to changes in pH is not due to a difference in concentration of ions inside and outside the cell, but is more likely due to changes occurring in the cells themselves, whether the membrane or the interior. It is suggested by McSwiney(7) that the varying tone of plain muscle is due to a changing distribution of the concentration of water in the phases of its colloidal systems. If this be so, then there is no reason why this change should not be induced by rapid changes such as are described and, with each variation in equilibrium, a condition of greater susceptibility to stimulation is left behind, thus accounting for the sensitising action

## SUMMARY

1 There is an increasing reaction of mammalian blood vessels to successive changes in hydrogen-ion concentration towards the alkaline side

2 An exaggerated response to adrenaline accompanies the reaction to alkalies

3 The plain muscle of the uterus and the small intestine gives similar results

I wish to express my thanks to Prof R J S McDowall for much kind assistance and advice I have received during this investigation

The expenses of this work were defrayed in part by a grant from the Government Grants Committee of the Royal Society, and I am also indebted to Parke, Davis and Co for supplies of adrenalin.

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## THE DIFFERENTIAL PARALYSIS OF CARDIAC NERVE ENDINGS AND MUSCLE

BY W R WITANOWSKI (*Fellow of the Rockefeller Foundation*)

(*From the Pharmacological Department, University College, London*)

O LOEWI<sup>(1)</sup> has shown that the inhibitory action of the vagus on the heart is associated with the liberation of a cardiac depressor substance, which has a pharmacological action on the heart similar to that of choline. Loewi and Navratil<sup>(2)</sup> also have shown that in the atropinised heart this inhibitor substance is still liberated when the vagus is stimulated, although the atropine prevents any apparent action upon the heart. The site of action of atropine therefore is the heart muscle and not the vagal nerve endings. Navratil<sup>(3)</sup> found that camphor had a double action in that it diminished the liberation of depressor substance from the vagal nerve endings, and also diminished the action of such substances upon the heart muscle. Loewi<sup>(1)</sup> has also shown that stimulation of the sympathetic nerves is associated with the liberation of an augmentor substance which resembles adrenaline in its action. The experiments described below were undertaken to try and find means of paralysing the vagus and sympathetic nerve endings in the heart without diminishing the response of the heart to choline compounds or to adrenaline.

*Method* The experiments were performed on the isolated heart-vagus preparation of *Rana temporaria*. A Straub cannula holding about 1 c c was used. The Ringer's fluid had the following percentage composition: NaCl 0.65, KCl 0.02, CaCl<sub>2</sub> 0.02, NaHCO<sub>3</sub> 0.02 (pH = 8).

In order to eliminate effects due to changes in frequency the ventricle in most experiments was driven at about 35 per minute by break induction shocks. The vagus was stimulated by induction shocks at a rate of 10 to 20 per second. The weakest effective stimulus was always employed.

*The Effect on the Vagus of Reduced Sodium Concentration* To study the effects of reduction in sodium chloride concentration a perfusion fluid was used containing NaCl 0.325 p c, cane sugar 3.4 p c, or glucose 1.7 p c, together with the other constituents of Ringer in normal con-

centration. This fluid is referred to as "half sodium Ringer." Acetylcholine was employed as a convenient vago-mimetic drug

Fig 1 shows the effect of changing from normal Ringer to half

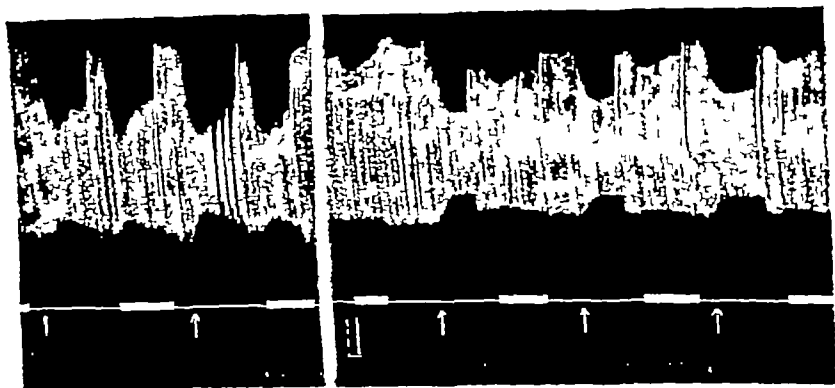


Fig 1 Effect of reduction of sodium chloride content of Ringer's fluid on the response of the heart to vagal stimulation and to acetylcholine. Artificial rhythm, 34 per min. The tracings show from above downwards. (1) Contraction of the heart. (2) Signal for vagal stimulation. (3) Time 5 secs. The arrows mark the introduction of acetylcholine  $10^{-9}$  molar. I. Normal Ringer (NaCl 0.65 p.c.) II. Ringer containing NaCl 0.325 p.c. and glucose 1.7 p.c.

sodium Ringer upon the response of the heart to vagal stimulation and to acetylcholine. With normal Ringer it was found that addition of  $10^{-9}$  molar acetylcholine produced the same reduction in amplitude of contraction as stimulation of the vagus with a weak current of known intensity and frequency. The figure shows that the immediate effect of changing to half sodium Ringer was to reduce very greatly the response to vagal stimulation (the current strength used being unchanged), while the response to acetylcholine was unaltered. Prolonged perfusion with half sodium Ringer gradually reduced the response to acetylcholine as well as the response to vagal stimulation. When the perfusion fluid was changed back to normal Ringer the activity both of the vagus and of acetylcholine were completely and rapidly restored.

This experiment was repeated fourteen times and on ten occasions results similar to those shown in Fig 1 were obtained, but on four occasions the two actions were affected equally.

Reduction in sodium chloride concentration reduces therefore both the activity of the vagus and the intensity of action of acetylcholine, but since it affects the former much more rapidly than the latter the two actions are partly separable.

*The effect on the sympathetic of reduced sodium concentration* The fact that reduction in sodium concentration reduces the effect of vagus stimulation more than the effect of acetyl choline might possibly be due to the change in sodium concentration making the sympathetic fibres more easily excitable

The effect produced on the sympathetic by reduction in sodium concentration therefore was tested. The hearts were atropinised and the vago-sympathetic trunk stimulated. The heart was allowed to beat with its normal rhythm but otherwise the experimental conditions were unaltered. Fig 2 shows a typical result. In this case stimulation of the vago-sympathetic trunk produced a 20 p.c. acceleration while adrenaline  $10^{-5}$  molar produced a similar effect. The following percentage accelerations were observed with varying molar concentrations of adrenaline  $10^{-4}$  molar — 62,  $10^{-5}$  molar — 27,  $10^{-6}$  molar — nil

Fig 2 shows that introduction of half sodium Ringer practically

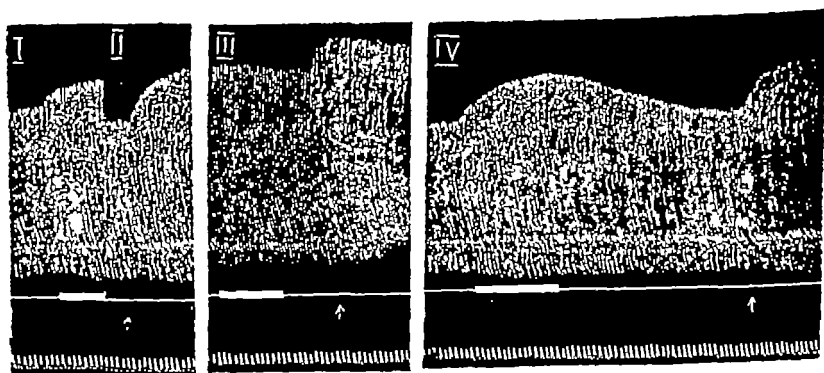


Fig 2 Effect of reduction of sodium chloride content of Ringer's fluid on the response of atropinised heart to sympathetic stimulation and to adrenaline. Tracings as in Fig 1. Natural rhythm. Arrows mark the introduction of adrenaline  $10^{-5}$  molar. I and II normal Ringer, III Ringer containing NaCl 0.325 p.c. and cane sugar 3.4 p.c. IV Normal Ringer. The acceleration in frequency produced was as follows

	Sympathetic stimulation	Adrenaline $10^{-5}$ molar
I and II	27	20
III	3	34
IV	17	33

abolished the response to vago-sympathetic stimulation but did not affect the response to adrenaline. A return to normal Ringer completely restored the response to vago-sympathetic stimulation. A reduction in sodium chloride concentration therefore depresses the activity of the

sympathetic nerve endings in the same manner as it depresses the vagal endings

*The action of ether* Ruttgers(4) found that vagal stimulation produced no effect on the heart in the presence of 0.25 p.c. ether. I tested the effect of ether on the response of the heart to vagal stimulation and to acetyl choline. The experimental method was the same as that previously described except that the volatility of ether necessitated frequent changes of the fluid in the Straub cannula.

The effect of 0.25 p.c. ether by volume is shown in Fig. 3. This concentration of ether produced no measurable reduction in the amplitude of contraction of the heart. The heart in Fig. 3 had been perfused for 24 hours and was in a hypodynamic condition and the amplitude actually increased after the addition of ether. This did not happen with the fresh heart but even in this case no certain and permanent reduction in the force of contraction occurred with 0.25 p.c. ether.

In Fig. 3 acetyl choline  $2.53 \times 10^{-8}$  molar produced the same depression as vagal stimulation in normal Ringer, but the addition of



Fig. 3 The influence of ethyl ether on the response of the heart to vagal stimulation and to acetyl choline. Tracings as in Fig. 1. Artificial rhythm, 29 per min. The arrows mark the introduction of acetyl choline  $2.5 \times 10^{-8}$  molar. I. Normal Ringer. II. Ethyl ether 0.034 molar introduced just previously. III. 5 mins. later. IV. 10 mins. after III. Ether present in II, III and IV.

0.25 p.c. ether at once abolished the effect of vagal stimulation whereas the action of acetyl choline was at first unaffected but it also was abolished after about ten minutes' exposure to ether.

The action of ether therefore resembles that of reduction in sodium chloride in that in both cases the vagus is paralysed rapidly but the action of acetyl choline is reduced much more slowly. A similar effect was produced by 1 p.c. ether, I found however that, as Ruttgers(4) stated, ethyl and methyl alcohol in moderate concentrations did not paralyse the vagus.

*Discussion* These experiments show that it is possible to produce

*The effect on the sympathetic of reduced sodium concentration* The fact that reduction in sodium concentration reduces the effect of vagus stimulation more than the effect of acetyl choline might possibly be due to the change in sodium concentration making the sympathetic fibres more easily excitable

The effect produced on the sympathetic by reduction in sodium concentration therefore was tested. The hearts were atropinised and the vago-sympathetic trunk stimulated. The heart was allowed to beat with its normal rhythm but otherwise the experimental conditions were unaltered. Fig 2 shows a typical result. In this case stimulation of the vago-sympathetic trunk produced a 20 p.c. acceleration while adrenaline  $10^{-5}$  molar produced a similar effect. The following percentage accelerations were observed with varying molar concentrations of adrenaline  $10^{-4}$  molar — 62,  $10^{-5}$  molar — 27,  $10^{-6}$  molar — nil

Fig 2 shows that introduction of half sodium Ringer practically

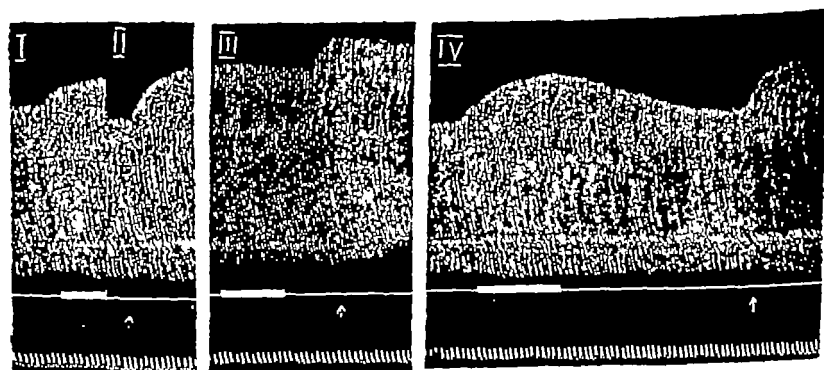


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*Discussion* These experiments show that it is possible to produce

a condition in the heart in which the vagus and sympathetic nerves are paralysed although the response to acetyl choline and adrenaline are unaffected In the experiments described the condition was a transitory one for the response to the drugs mentioned was slowly diminished I was not able to find a drug which would paralyse the vagus or sympathetic and would leave the response to acetyl choline and adrenaline permanently unaffected

The results are in accordance with Loewi's view that the action of the vagus or sympathetic on the heart consists of two separable stages, namely, (1) the liberation of some active substance by the nerve endings, and (2) the action of these substances upon the muscle cells

### CONCLUSIONS

(1) Either a reduction of the sodium chloride content of Ringer's fluid to one half, or the presence of 0.25 p.c. ether, paralyses the vagi more rapidly than it abolishes the effects produced by acetyl choline

(2) A reduction of the sodium chloride content of Ringer's fluid to one half paralyses the sympathetic more rapidly than it affects the response of the heart to adrenaline

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# THE RECOVERY HEAT PRODUCTION IN TORTOISE'S MUSCLE

By W HARTREE<sup>1</sup> AND G LILJESTRAND

*(From the Physiological Laboratory, Cambridge,  
and University College, London )*

SINCE the recovery heat production, already determined in the case of the frog by Hartree and Hill(1), is of fundamental importance in any theory of muscular contraction, it was evidently desirable to find if there was any difference in this respect between the quickly responding frog's muscle and the much more slowly responding one of the tortoise. For this purpose two separate series of experiments have been performed, in each of which the recovery heat in the biceps cruris of a tortoise kept in oxygen was determined, a slowly acting thermopile being used in either case in order to ensure that the maximum deflection of the galvanometer was correctly proportional to the total initial heat, even for a long stimulus of 2 seconds or more. The actual deflections were read on a scale, photographic recording being unnecessary owing to the slow movement of the galvanometer. In general the method employed by Hartree and Hill(1) was followed.

Two principal points were investigated: firstly, the total amount of the recovery heat as compared with the initial heat, and secondly, the rate at which the recovery process takes place under different circumstances.

The first series of experiments (carried out by G. L.) was principally concerned with the first point, and for this purpose it was necessary only to measure the whole area of the curve between galvanometer-deflection and time consequent on a stimulus, and to compare this with the whole area of the control curve (the dead muscle being heated electrically for a short time). For the same maximum the area of the control curve represents the initial heat on the same scale as the area of the live curve represents the total heat, i.e. initial plus recovery heat. In this series of experiments the temperature was from 16° to 18° C. and since it was found that a short stimulus gave rise only to a very small initial heat, the time of stimulus was always about 2 seconds. Even then the galvanometer had to be made rather sensitive (about 30 mm. per microvolt at

<sup>1</sup> Working for the Medical Research Council.



1 metre distance) so as to obtain suitable deflections. The results for the ratio of total heat to initial heat in 22 observations on eight different animals were 1.8, 1.8, 1.8, 1.9, 2.0, 2.0, 2.1, 2.1, 2.1, 2.1, 2.2, 2.3, 2.3, 2.3, 2.3, 2.4, 2.4, 2.5, 2.5, 2.6, 2.7, 2.7, with an average of 2.2, giving the recovery heat 1.2 times the initial heat. In consequence, however, of a small deflection persisting after 10 minutes it must be admitted that the results should probably be rather greater.

In this series a few observations were made on the delayed heat when the muscle was in nitrogen after previous soaking for at least 30 minutes in Ringer's solution which had been boiled to render it free from oxygen. Four determinations gave the delayed heat as 0.25-0.30 of the initial heat, as found by Hartree and Hill for frog's muscle (2). An analysis, however, of the gas in the muscle chamber showed that there was 1 or 2 p.c. of oxygen still present, so that no doubt the actual delayed heat in pure nitrogen would be somewhat less than the above figure.

The second series of experiments (carried out by W. H.) extended over several months, a large number of observations being required in order to obtain a good average, in view of the variability of the individual specimens examined (see (3) and (4)). In this series many analyses of curves of galvanometer deflection by means of the corresponding control curves have been made so as to determine the rate of heat production throughout the recovery process, the total recovery heat also was found in every case.

In this series the temperature was from 18° to 20° C, the average for all experiments being very nearly 19° C. The times of stimulus employed were 2.0, 1.0 and 0.5 sec, but for the last of these it was found that the rate of recovery was so small and so prolonged that it was impossible to make a good estimate of the total. Of this only about two-thirds had taken place up to 10 minutes after the stimulus. For the other times of stimulus the average values of the ratio of recovery to initial heat are 1.45 for 1 second and 1.75 for 2 seconds, for about 12 reliable experiments in each case. It should, however, be remarked that the value of this ratio in March and August seems to be consistently greater than in April and May, and that more experiments with 2 seconds' stimulus were performed in the former months than with 1 second's stimulus. This also may explain the still lower value (obtained by G. L.) in the first series of experiments which were performed in May and June. Thus, on the whole, the average value of this ratio is evidently very nearly 1.5, as in the case of frog's muscle. This is very satisfactory, as showing the inherent similarity of the recovery process in the two cases.

As regards the course of the recovery process, Fig. 1 shows the relative rate of heat production ( $\dot{q}$ ) expressed throughout as a fraction

of the total initial heat) for 10 minutes after the stimulus, in the same manner as in the case of frog's muscle by Hartree and Hill (1), p 370)

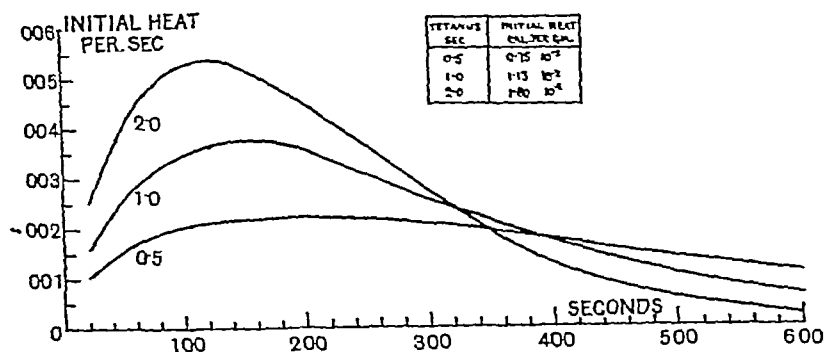


Fig 1 Curves showing the relative rate of recovery heat production for three different times of stimulus 0.5 1.0 and 2.0 seconds Tortoise muscle in oxygen at 19° C The heat unit for each curve is the corresponding initial heat as entered in the small table in the figure The curves for 2.0 and 1.0 seconds are the mean of four or five very similar curves from different experiments. That for 0.5 second is less reliable since only a few observations were made with this time of stimulus A few observations with stimulus 0.1 sec. showed an even lower curve, but a case with a much longer time of stimulus (4 seconds) did not appear to give a curve rising much above that for 2 seconds

The curves are of the same general nature, those for the longer stimuli rising higher and having earlier maxima and falling more rapidly than those for shorter stimuli In the present case the maximum relative rate of heat production is closely proportional to the initial heat per gram of muscle, or, expressed in another way, the maximum actual rate of recovery heat production in calories per gram per second is closely proportional to the square of the absolute initial heat per gram Expressed in a formula

$$(\text{maximum recovery rate}) = 0.32 (\text{initial heat})^2$$

The corresponding factor for the case of frog's muscle at 20° C is about 0.48

Thus, for the same absolute amount of recovery, the curves for a tortoise and a frog are not far different, those for a tortoise being rather lower with a later maximum and falling slightly more slowly than in the case of a frog at the same temperature They might be identical with those for a frog at a slightly lower temperature The very great difference between the two cases is that it requires about 15 times as long a stimulus in the tortoise's muscle as it does in the frog's to produce the same initial heat per gram In spite, therefore, of the great difference in

speed of the initial process between the muscles of tortoise and frog, the recovery processes are in type exactly similar and occur for a given initial heat at almost the same rate no stronger testimony could be desired as to the essential biochemical similarity of the recovery processes in the two cases

The curves of Fig 1 are not continued back to the origin the reason for this is that the initial process in tortoise's muscle is relatively so slow that the difference between the curve of galvanometer deflection after a stimulus and the control curve (which is what is analysed to give the recovery heat) is not reliable for the first 20 seconds or so In the majority of cases, however, the rate of recovery increased from an initial low value occurring soon after the stimulus In several experiments this was not the case, especially for the first one or two stimuli, although the curves of Fig 1 were generally approached with later stimuli This occasional initial abnormality was sometimes so large that it could not have been due to experimental error, and in such cases it should be mentioned that it is impossible to determine the total recovery heat since the first part of it becomes involved with the initial heat A few examples are shown in Fig 2

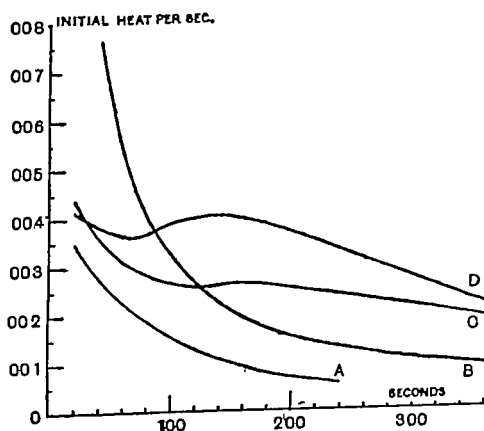


Fig 2 Curves showing abnormal type of recovery heat production soon after the stimulus Tortoise's muscle in oxygen at  $19^{\circ}\text{C}$  A, B and C are the first records taken in their respective experiments (after one or two preliminary stimuli) with 1 second stimulus D was for a 2 second stimulus, and this type of curve persisted throughout the experiment.

A Total delayed heat 0.5 times initial heat, for this, the first record For the second record 0.75, etc Later records taken after an interval

of 1 hour were quite normal, as in Fig 1, the recovery heat certainly starting off from a low value

*B* Starts at a very high rate, apparently several times the normal maximum rate of recovery heat production

*C* and *D* It seems impossible to avoid the conclusion that these arise from the combination of a curve of type *A* with one of the normal type shown in Fig 1, but it should be remarked that the total area in these cases is not excessive since the subsequent maximum when it occurs is lower than the normal (compare Fig 1)

Thus, heat production occurring early after the stimulus seems to be at the expense of subsequent recovery heat and all the curves *B*, *C*, *D* are of normal area approximating 1.5, although no sign of the normal recovery curve can be detected in *B*

In a few cases the slight fall soon after the start, as in curve *D*, was maintained throughout all the records of one experiment For this reason also it was necessary to do a large number of experiments so that the normal rate could be well determined We are unable to explain the abnormalities which must be left for subsequent investigation

#### SUMMARY

1 The initial processes in tortoise's muscle occur much more slowly than in frog's muscle It was of interest, therefore, to compare the recovery heat production in the two cases

2 The recovery heat averages about 1.5 times the initial heat, exactly as in the case of frog's muscle

3 For a given duration of stimulus the recovery heat production in tortoise's muscle occurs much more slowly than in frog's muscle This, however, is due almost entirely to the fact that the initial heat for a given duration of stimulus is much less in tortoise than in frog For a given initial heat production the rate of recovery heat is nearly as great for tortoise as for frog

4 The time course of the recovery heat is entirely similar in the two cases, the maximum rate of recovery being proportional to the square of the total initial heat, suggesting some kind of bimolecular reaction

5 Certain abnormalities in the recovery heat are discussed

The expenses of this research have been borne in part by a grant from the Royal Society

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# THE "INTERFERENCE" OF TWO FLEXION-REFLEXES, AN EXAMINATION OF VÉSZI'S EXPERIMENT BY T GRAHAM BROWN

(From the Physiology Institute, Cardiff)

IN 1910 VÉSZI(1) described an experiment which has been taken to have an important bearing on the problem of central inhibition

The experiment is as follows

Reflex contraction of *m. gastrocnemius* in the frog may be elicited by repetitive faradic stimulation applied to the central cut end of each of several posterior spinal roots, (*e.g.* VIII, IX, X) If, however, during a long continued stimulation of root IX, root VIII or X (*e.g.*) is concurrently stimulated, the "background" tetanic contraction of *gastrocnemius* is replaced by inhibitory relaxation—although the interrupting stimulus evokes reflex contraction of the muscle when given alone This observation has been used—for example by Keith Lucas (3, p 96)—as an indication that an inhibitory effect is produced by the "increased frequency of the impulses which would reach the final common path when both roots are stimulated at once"

The hypothesis is that central inhibition occurs where nerve-impulses follow one another at too great a frequency Each then falls in a preceding relative refractory phase Each is thus so reduced in magnitude that it is unable to pass through a succeeding place of decrement Vészi's observation has been accepted as experimental support for this hypothesis Verworn (2), p 225 explains Vészi's experiment as an instance of inhibition due to "interference"

Vészi's observation is, however, capable of another explanation His interrupting stimulus evoked a reflex contraction of *gastrocnemius* which did not continue for the whole period of repetitive stimulation This "Anfangstetanus" is a twitch-like contraction of the muscle, and gives place immediately to relaxation which continues during the remainder of the period of stimulation

Vészi did not record the synchronous movements of an antagonistic muscle (flexor), and it is therefore impossible now to know what actually occurred in the flexors during the later part of the interrupting stimulation It is more than probable that his interrupting stimulus evoked a di-phasic reflex—the first phase represented by the brief extensor contraction which he recorded, and the second phase consisting in a contraction of the flexors (which he did not record) In this connection it is important to note that Beritoff(4), in recording flexors and extensors simultaneously, found di-phasic reflexes on stimulation of the

central cut end of posterior root X in the frog. He found that sometimes extension occurs first and is followed by flexion, at other times this order is reversed. Beritoff points out that this posterior root contains afferent fibres from the receptive fields of functionally different reflexes, and suggests that the di-phasic effect of stimulating a mixed root (or a mixed afferent nerve) is due to combination of the various reflexes evoked by stimulation of the different functional groups of afferent fibres which run in it.

*The present observations.* On the supposition that Vészi's result is really due to a di-phasic response evoked by the interrupting stimuli he used, a parallel observation may be made in the decerebrate cat where a di-phasic flexion-extension reflex is compounded against a "background" of maintained flexion. The chief difficulties presented in searching for these conditions are the comparative rarity of reliable di-phasic reflexes and the comparative difficulty of obtaining reflex inhibition of flexion. In a recent experiment the proper conditions were obtained.

Black female cat, 1730 gm., May 14th, 1926. Preparation started 10.35 a.m. Decerebration performed 11.15 a.m. at end of all operative procedure. Chemical narcosis had been stopped just before decerebration.

The cat was prepared for graphic isotonic registration of tibialis anticus and gastrocnemius soleus of both right and left sides of the body. Remaining muscles of both hind limbs were paralysed by tendon or motor nerve section. Cat fixed in position for recording about mid-day. Needle electrodes inserted under skin of both hind paws for reflex stimulation. The sacrum, femora, and tibiae firmly clamped in such a posture that the spinal axis was parallel to the floor with the dorsum of the cat up. The fore part of the cat swung loosely from the ceiling with the fore paws clear of the table. The front part of the cat could therefore take up any posture or perform movements freely.

This particular preparation was an extremely stable one and gave very regular results for 33½ hours after decerebration—the last records (taken at 9.0 p.m. on May 15th) showing little apparent deterioration. The preparation died during the night of the 15–16 May. The fore part of the cat hung symmetrically throughout the whole period of the experiment—there being no asymmetrical posture, such as is sometimes seen where bleeding occurs from the decerebration wound during an experiment. The results to be described were obtained on the morning of May 15th during the period from about 22½ hour to about the 25th hour after decerebration.

### *Results*

The following instance only need be described.

#### *Right limb results*

A (Fig 1) Repetitive faradic stimulation of the right hind paw at 2000 Berne units (frequency about 20 per second, primary current, 0.5 amp), continued for 12 seconds, evokes a maintained reflex con-

traction of right tibialis anticus The plateau of contraction is attained about  $2\frac{1}{2}$  seconds after the commencement of stimulation and falls slightly during the remaining period

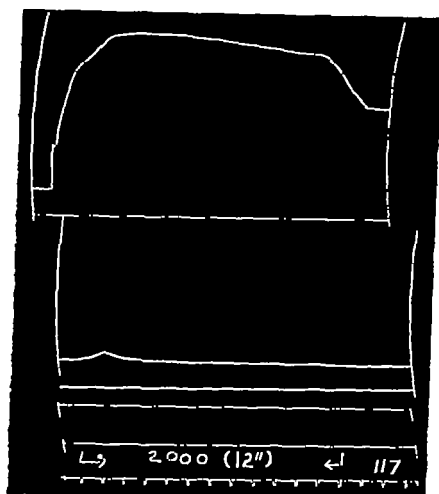


Fig 1 The "background" right ipsilateral flexion reflex—described as "A" in text (No 15216 obtained 23 hours 52 minutes after decerebration) This reaction was taken 45 minutes before the reactions illustrated in Fig 2 The flexor contraction is not so well maintained as it was in a similar record obtained 28 minutes after the reactions in Fig 2

In this and the succeeding figure top myograph line = isotonic reflex movement of right tibialis anticus  $\times 2^1$ , lower myograph line = isotonic reflex movement of right gastrocnemius soleus  $\times 4^1$ —in either case an arbitrary base line is drawn underneath the myogram during the experiment. Upper signal line = commencement (fall of signal) and termination (rise of signal) of repetitive faradic stimulation of left hind paw Lower signal line = the same for right hind paw Lowest line = time in seconds

NOTE The stimulus signal failed during this record, but the stimulus—which lasted 12 seconds—did not fail The exact times of commencement and termination of stimulus are immaterial, the approximate points are marked.

B (Fig 2) Repetitive faradic stimulation of the left foot at 2500 Berne units (frequency about 20 per second, primary current 0.5 amp), continued for 2 seconds, evokes a brief contraction of right flexor which gives way to relaxation during stimulation Relaxation is complete about 1.2 seconds after the commencement of stimulation Almost synchronously with the attainment of flexor relaxation, right gastrocnemius rises in a well-marked contraction which persists as after-

<sup>1</sup> I.e. in original record as here reproduced, tibialis anticus myogram shows the true shortening and lengthening of muscle, gastrocnemius soleus myogram shows movement  $\times 2$

discharge on termination of the stimulus. The reaction is therefore markedly di-phasic, and the record of the flexor muscle strictly comparable to Vészi's records of gastrocnemius in the frog

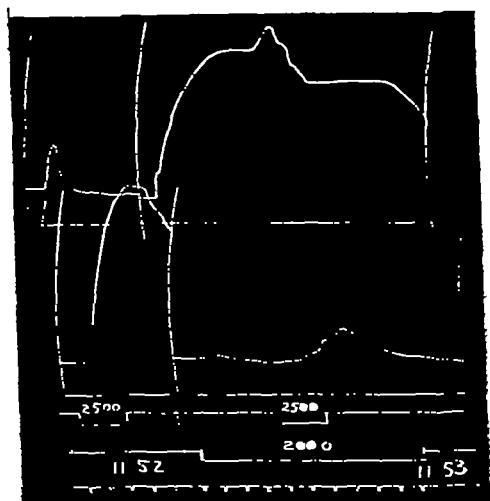


Fig 2 Two reflexes from the same preparation (Nos. 15251, 15252 obtained 24 hours 38 minutes after decerebration) The first reaction is that described as "B" in the text. It consists in a di phasic reflex commencing with flexion and reversing to extension during stimulation, the extension being continued after stimulation as after discharge. The second reaction—described as "C" in the text—is obtained by compounding the immediately preceding reaction with the "background" illustrated in Fig 1. There is algebraic summation of the two phases of "B" against the steady flexion of "A."

C (Fig 2) Reaction B made to interrupt "background" A, the commencement of the interrupting stimulus falling 4 seconds after the commencement of the "background" stimulus

The first effect seen after the commencement of the interrupting stimulus against the "background" is an augmentation of reflex flexor contraction. This gives place to relaxation during the period of compound stimulation and at the end of the 2 seconds of the interrupting stimulation the level of flexor contraction is considerably lower than the plateau of the simple "background" flexion. This depression persists, after the interrupting stimulus is stopped, for the remaining 4 seconds during which the "background" stimulus is continued alone.

The extensor exhibits reflex contraction in correspondence with the relaxation of the flexor muscle during the later part of the interrupted stimulus, and this contraction becomes augmented after the withdrawal of the interrupting stimulus.



Comparison of the time relations of the movements in the simple interrupting reflex and the interrupted period of the compound reflex shows (a) the maximum of flexor contraction in the latter occurs later than in the former (say 0.2-0.25" later), (b) the subsequent flexor relaxation also occurs later in the compound reflex, and the relaxation is slower. These observations, together with the clear augmentation of flexor contraction in the compound reflex, point to an algebraic summation of the two simple flexions and not to an "interference inhibition" of flexion.

The experimental result is this

The interrupting di-phasic stimulus (flexion followed by extension) gives a similar di-phasic effect when compounded against a "background" reflex of maintained flexion. There is algebraic summation of the two parts of the di-phasic reflex with the "background" flexion.

#### *A possible explanation of Vészi's experiment*

Graphic reproductions of Vészi's experimental results are contained in his original paper<sup>(1)</sup>, and other examples are illustrated by Verworn<sup>(2)</sup>, pp 43, 224, 225 and 226)

If Vészi's result is to be taken to show that two stimuli, each of which gives reflex contraction of the same muscle, may give inhibitory relaxation when compounded together, the important phase in the compound reflex is that which corresponds in time with the brief "Anfangstetanus" evoked by the interrupting stimulus when it is given alone. The analysis of the records quoted above is rendered difficult by the absence of ordinates such as are made on the stationary drum at the commencement or termination of the tracings. But there is sufficient internal evidence<sup>1</sup> to show that the inhibitory relaxation obtained by Vészi when he stimulated the Xth root during maintained reflex contraction evoked from the IXth root occurs *later* in time after the commencement of the interrupting stimulus than (or at least as late as) the relaxation in the "Anfangstetanus" evoked when the interrupting root is stimulated alone.

There is thus no reliable evidence that the inhibitory relaxation of gastrocnemius seen during the interruption of the IXth root reflex by Xth root stimulation is due to "interference" of the "Anfangstetanus" stimulus with the "background" stimulus. It is as probable, or more probable, that the inhibitory relaxation of the compound reflex is strictly comparable to the relaxation which terminates the "Anfangstetanus" obtained on simple stimulation of the Xth root.

<sup>1</sup> See the graphic tracings figured by Verworn ((2), Fig 58, p 225)

The suggestion may be made that the relaxation of the "Anfangs tetanus" was accompanied by reciprocal contraction of the flexor muscles (not recorded by Vészi) and that the whole phenomenon may be quite simply explained if Vészi evoked a di-phasic reaction on stimulation of the "interrupting" root. In this case the (first) extension phase of the di-phasic reflex sums with the extension of the "background" reflex, and the (second) flexion phase of the di-phasic reflex (consisting of excitation of the flexor moto-neurones and inhibition of the extensor) sums algebraically with the "background" extensor contraction. During this second phase, the extensor inhibition of the di-phasic reflex gives the extensor relaxation of the "background" gastrocnemius contraction seen in the compound reaction.

The results described here in the decerebrate cat give strong support to this view. If the flexor muscle alone is examined, the records are similar to those obtained by Vészi (with an extensor). The extensor muscle gives the additional information pointing certainly to the di-phasic nature of the phenomenon. In the first period after the interrupting stimulus has been put against the "background," there is algebraic summation of the (first) flexion phase of the di-phasic interrupting reflex with the "background" flexion, in the second period there is algebraic summation of the (second) extension phase of the di-phasic reflex with the same flexion "background."

#### SUMMARY

1 Vészi's experiment has been interpreted to mean that two series of repetitive stimuli, each of which are excitatory for the extension reflex in the frog, may when applied synchronously give an "interference" effect resulting in inhibition of reflex extension.

2 A comparable experiment in the decerebrate cat gives no evidence that this is the case. On the contrary, algebraic summation occurs.

3 There are good grounds for supposing that Vészi's result is due to the algebraic summation of each of the succeeding extension and flexion phases of a di-phasic reflex with a "background" of maintained extension when the di-phasic reflex is used to interrupt a maintained extension-reflex.

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# THE APPLICATION OF THE ETHYL IODIDE METHOD TO THE DETERMINATION OF THE CIRCULATION RATE IN WOMEN

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DURING the war, the late Prof Brodie initiated some work on the effects of penetrating chest wounds upon heart output. The work was carried out by Prof Brodie and one of us (W C C) in conjunction with Mrs Oppenheimer and the late Prof J J Mackenzie, and was made possible by the facilities kindly given to us by Dr Louisa Garrett Anderson and the late Dr Flora Murray at the Endell Street Military Hospital.

The determinations of output were made by a modification of Krogh's nitrous oxide method<sup>(1)</sup> and observations extended over a period of more than 18 months. The results were not published, since great discrepancies occurred in the figures obtained for any one individual, even when there was, so far as could be seen, no obvious failure in the technique of the actual experiments. In many cases the results justified the view that a curtailment of the exchanging surface of the lungs was compensated for by an increased flow through the active part of the lungs as measured by the gas absorption, and probably also by an increase in the heart output. The true extent of the increase would obviously not be given by this method based upon respiratory exchanges if there were still a circulation through the collapsed lung tissue. Observations on normal individuals gave an average circulation rate of 4-5 litres or even less, whilst with the wounded, rates of 7, 8 or 9 litres were more often obtained. But, even in intelligent subjects giving the greatest possible co-operation and on whom observations usually gave average results, circulation rates of 3 or 12 litres might occasionally be obtained from experiments otherwise apparently satisfactory. For this reason it was not possible to draw any definite conclusions as to the

effects of chest wounds upon circulation rate and the investigation was finally abandoned after considerable work on normal individuals had convinced us that in our hands this method, for various reasons could not be relied upon to give consistent results

More recently, work on heart output was again undertaken, and was still further extended, owing to the award to one of us (O R) of a research scholarship to follow through the heart changes in pregnancy, in subjects suffering from heart lesions. Careful consideration of the problem led to the conclusion that a study of heart output would best serve the purpose since this figure would give accurate information as to the added amount of work thrown on the heart in pregnancy. Our trial of various methods led us to the view that, though these might give reliable results upon subjects who could give intelligent co-operation, calling in some cases for considerable training and control, they were not suitable for use where whether from illness or lack of familiarity with the method, such co-operation could not be expected. The use of methods involving the taking of blood samples would also considerably limit the number of observations that could be made

A note in a paper by Prof. Yandell Henderson referring to the advantages of ethyl iodide in such studies led to communications with him, which resulted in our having the very great advantage of his help in setting up in our laboratories all the apparatus required for determination of heart output by this method. In our earlier observations we also had his co-operation in the actual experiments which saved us many hours in developing our technique

The present paper gives an account of a series of observations upon the circulation rate of normal women as measured by the ethyl iodide method(2). The experiments were made in such a manner as would, we thought, give every opportunity of testing the reliability and general ease of application of the method to persons with no particular experience of it, and further would give, if successful, information as to the circulation rate in a group of healthy women.

Determinations have been made under standard conditions with the subject in a semi-reclining position after a period of rest of not less than 30 minutes. In most cases the subject had a light breakfast of tea and toast 1-1½ hours before making the determination.

A few estimations were made after lunch when an interval of 2 hours was allowed. The effect of digestion on the cardiac output will be referred to later, but it appears that even after a heavy meal, the circulation rate is back approximately at the resting level within 2 hours. The actual

duration of the experiment must be at least 10 minutes, so as to allow time for the alveolar sampling tube to be completely washed through with alveolar air. The oxygen consumption is estimated each time by collecting a sample of expired air in a Douglas bag, during the latter half of the experiment. This also forms an additional check on the volume of air breathed.

Table I gives the results of a series of experiments performed under the standard conditions on two individuals.

TABLE I.

Subject *E D* age, 22, weight, 60 kg, height, 174 cm., average ventilation 5.22 litres per min., average  $O_2$  consumption, 225 c.c. per min.

Date	Pulse	Circulation litres per min.	Stroke vol. c.c.	Stroke index c.c. per kg
20 x 25	64	9.20	144	2.40
20 x 25	74	8.48	87	1.45
20 x 25	66	6.25	95	1.58
30 x 25	63	8.75	139	2.31
2 ii 26	65	5.80	89	1.48
2 ii 26	66	6.60	100	1.67
3 ii 26	64	6.75	105	1.75
3 ii 26	67	7.60	113	1.88
3 ii 26	72	8.00	111	1.85
4 ii 26	64	6.40	100	1.67
Average	66	7.18	108	1.81

Subject *M E* age, 20, weight, 63 kg, height, 162 cm., average ventilation, 4.45 litres per min., average  $O_2$  consumed, 210 c.c. per min., athletic, rower.

Date	Pulse	Circulation litres per min.	Stroke vol. c.c.	Stroke index c.c. per kg
5 ii 26	84	5.20	63	1.00
16 ii 26	68	7.60	112	1.78
23 ii 26	78	11.50	147	2.34
2 iii 26	86	13.50	157	2.50
2 iii 26	82	10.50	128	2.03
9 iii 26	77	10.50	136	2.18
9 iii 26	70	10.40	148	2.35
Average	79	10.00	127	2.03

The observations extended over a period of several months. It will be seen that, on the whole, the figures are in close agreement with one another. In both the examples given the later results agree more closely than the early ones. This may be attributed partly to our improved technique and partly to the fact that the subject becomes more accustomed to the procedure, the first one or two experiments on any individual are always apt to be unreliable. Other reasons for a certain degree of variation are the unavoidable experimental error, which is probably about 10 p.c., and the difficulty of obtaining exactly similar

conditions, as is shown by variations in the oxygen consumption and pulse rate

The most constant figure for any given subject is obviously the stroke index, and if the earlier experiments recorded in this table are excluded, it will be seen that the later figures do not vary by more than plus or minus 10 p c from the average

In the case of *M E* the first estimation is printed in italics, as it is obviously too low as judged by her average figures, especially the stroke index. In this experiment the respiration rate was 20, whereas the usual rate for this subject is 6<sup>1</sup>. The total ventilation was not increased and the tidal air was therefore very low. In our experience this always tends to give a low circulation rate. The second experiment shows a slight improvement, the respiration rate being in this case 12.

Similar observations have been made on a group of students over a period of three months, and, whilst Table I gives two such sets of experiments in detail, Table II gives the average results obtained from

TABLE II

	Subject	No of experi- ments	Pulse	Circulation litres per min.	Stroke volume c c.	Stroke index c c per kg
<i>M E</i>	Age, 20 weight, 63 kg	7	79	10 00	126	2 00
<i>C C</i>	Age, 21, weight, 58 kg	3	76	8 21	108	1 86
<i>M W</i>	Age, 24 weight, 51 kg	4	90	8 40	94	1 84
<i>E D</i>	Age, 22, weight, 60 kg	10	66	7 18	108	1 80
<i>O R</i>	Age, 36 weight, 49 kg	11	71	6 30	88	1 81
<i>R S</i>	Age, 25, weight, 70 kg	4	64	7 80	122	1 75
<i>D B</i>	Age, 19, weight, 62 kg	4	61	8 50	106	1 70
<i>B J</i>	Age, 23, weight, 73 kg	4	72	8 78	122	1 67
<i>M R</i>	Age 23 weight, 48 5 kg	2	67	5 20	78	1 60
<i>J B</i>	Age, 21 weight, 65 kg	6	108	10 40	96	1 50
<i>M C</i>	Age, 20, weight, 58 kg	8	72	6 76	94	1 64
<i>J C</i>	Age, 19, weight, 61 kg	5	73	5 10	70	1 15
Average for 11 women, ages 19-25 (omitting <i>O R</i> )		57	75	7 5	100	1 67

all experiments on 12 subjects. These experiments are not specially selected, and only those results which are unsatisfactory on account of obvious technical defects, or of very low carbon dioxide percentages in the alveolar air, have been excluded.

Comparison of these results with those published by Henderson and Haggard shows that our figures, though giving slightly higher values, are, on the whole, in close agreement with theirs. Their figures for women

<sup>1</sup> We wish to draw attention to the fact that the average respiration rates observed in the majority of our subjects during these experiments are considerably below those normally accepted but are very constant for each individual.

are, however, taken from a few experiments only, and the ages range from 17 to 50 instead of from 19 to 25 as in our series

Details of our observations on one of the subjects are given in Table III, as the figures obtained show some features of special interest

TABLE III.

Subject *J B* age, 21, weight, 65 kg, height, 162 cm., persistent over breathing and rapid pulse during all experiments, normal resting pulse, 74, alveolar CO<sub>2</sub> (Haldane Priestley), 5.2 p.c., vital capacity, 3750 c.c. No evidence of hyperthyroidism

Date and time	Pulse	Circulation litres per min.	Stroke volume c.c.	Stroke index c.c. per kg	Ventilation litres per min	Oxygen consumption c.c. per min	Alveolar CO <sub>2</sub> p.c.*		
							M.V.	H.P.	
8 II. 26									
10 a.m.	106	11.50	109	1.68	9.80	291	3.21	3.71	
14 II. 26									
10 a.m.	118	14.30	120	1.87	12.20	291	3.64	—	
1.15 p.m.	118	11.75	100	1.54	10.40	264	3.64	—	
22 II. 26									
10 a.m.	110	10.10	92	1.42	12.00	281	3.41	3.63	
1 III. 26									
10 a.m.	100	8.50	85	1.30	9.50	213	3.67	3.76	
1.15 p.m.	100	8.00	80	1.23	10.80	232	3.52	3.69	

\* M.V. is the alveolar sample taken from the Müller valve of the apparatus and H.P. is the sample taken by the Haldane Priestley method.

In this subject, *J B*, the circulation rates obtained in the earlier experiments are abnormally high, although the stroke volume and stroke index are well within the normal limit. This is partly due to the rapid pulse rate which is never below 100. The main feature in this case, however, is the persistent over-breathing. In one experiment, which has not been recorded here, the ventilation was very uneven throughout, averaging 14 litres per minute, and at one period reaching the very high figure of 17 litres, the alveolar carbon dioxide being reduced to 2.7 p.c. In spite of this, she appeared to suffer very little discomfort beyond a marked flushing of the face, and there was no succeeding period of apnoea. The resistance of the valves was not more than 2 cm. of water and has been the same in all our other experiments. There is no marked increase of metabolic rate in this subject nor any other evidence of hyperthyroidism, the normal resting pulse rate being 74. We conclude, therefore, that the phenomenon is a purely nervous one.

A few observations have been made on some of our subjects to show the effect of food and exercise on the circulation rate. Table IV is included to show the type of result obtained, but the work on this point is as yet incomplete.

TABLE IV

Subject and conditions of experiment	Date	Pulse	Circulation litres per min.	Stroke volume c.c.	Stroke index c.c. per kg	Oxygen consumption c.c. per min.
O R age, 36, weight, 49 kg						
Average resting values		71	6.3	89	1.81	200
1 hour after lunch	12 XI. 25	78	8.4	108	2.20	260
1 hour after heavy lunch	4 II. 26	71	9.1	128	2.62	207
1 hour later	4 II. 26	70	5.9	84	1.71	168
1½ hours after lunch	24 II. 26	68	9.8	144	2.94	183
Exercise stationary running for 4½ mins, ½ hours after food	31 III. 26	144	18.2	126	2.57	1530
W L male, age, 39, weight, 70 kg	3 II. 25					
After 2½ hours fasting		64	7.05	110	1.57	267
2 hours later, 1 hour after meal	3 II. 25	80	11.70	146	2.09	328

*Practical details in application of method*

In view of the interest which the ethyl iodide method has aroused and of its possible clinical application, we think it may be useful to discuss some of the difficulties which we have encountered, in the hope that we may save time and trouble to others who are using the method. Points for discussion arise in connection with the following

- The alveolar air sample
- The estimation of ethyl iodide
- Pulse rate and total pulmonary ventilation.

(a) *The alveolar sample* We may remind readers that the calculation is made from the following formula

$$\text{Circulation} = \frac{\text{Ethyl iodide absorbed per minute}}{\text{Alveolar ethyl iodide concentration}} \times 2$$

The data necessary for this calculation are, therefore

- 1 The total pulmonary ventilation in litres per minute
- 2 The concentration of ethyl iodide in the inspired air
- 3 The concentration of ethyl iodide in the expired air
- 4 The concentration of ethyl iodide in the alveolar air

Of these, the only factor which offers any real difficulty is the last, and the success of the method largely depends on the extent to which the problem of obtaining a genuine sample of alveolar air has been solved. The same question arises in most of the other methods used for determination of the circulation rate, but in this particular one, the necessity for procuring a sample of at least 250 c.c. adds to the difficulty. Henderson and Haggard have shown that their method for collecting the



TABLE V Effect of tidal air on alveolar CO<sub>2</sub> percentages

Respir rate	Tidal air c c	Alveolar CO <sub>2</sub> percentages			Average alveolar CO <sub>2</sub> percentage
		Müller	Haldane	Priestley	
Subject <i>D B</i> Average circulation, 6.5 litres per min., stroke index, 1.70 c c per kg					
19	390	4.32	5.80	}	H. P 5.52 M. 4.15
17	335	3.59	5.45		
18	320	4.29	5.68		
18	370	4.39	5.17		
Subject <i>J C</i> Average circulation, 5.1 litres per min., stroke index, 1.18 c c per kg					
17	330	4.84	5.57	}	H. P 5.67 M. 4.66
17	310	4.73	5.64		
18	310	4.55	5.64		
20	330	4.48	5.81		
16	300	4.73	5.64		
Subject <i>M E</i> Average circulation, 10.0 litres per min., stroke index, 2.0 c c per kg					
5	660	6.01	5.89	}	H. P 5.81 M. 5.86
6	770	5.86	5.82		
6	750	5.85	5.62		
5	760	5.84	5.79		
5	920	5.73	5.94		
Subject <i>R S</i> Average circulation, 7.8 litres per min., stroke index, 1.75 c c per kg					
8	700	5.72	5.45	}	H. P 5.75 M. 5.83
7	700	5.97	5.62		
6	880	5.82	5.70		
8	500	5.81	6.16		

method from the British Drug Houses, Ltd, who are putting it up in Pyrex glass tubes ready for use

In the method as originally described, an oil bath is used as a means of maintaining the correct temperature of the iodine pentoxide tube, which should be 160°–200° C. This is a simple method and maintains a constant and even temperature. It has, however, one serious disadvantage. The pentoxide tube may break, and a violent reaction take place between the oil and the pentoxide. This accident occurred to us near the beginning of our work, and, although there was no actual explosion, enormous volumes of iodine were given off, sufficient to render the laboratory uninhabitable for several minutes, and the pentoxide tube was completely spoilt.

We have now, with the co-operation of Miss Waller, devised an electrically-heated air bath, and this has proved both simple and effective. There is no risk of an explosion and no need for a draught cupboard. The

apparatus consists of a box made out of asbestos and fastened together with paper clips. There is a slit in the lid through which the U-tube containing the pentoxide is passed, the tube hanging free in the box by means of its arms which rest on the outside of the lid. These are packed round with asbestos wool and a further cover put over the whole to prevent cooling and consequent deposition of iodine. The sides of the box are lined with two woven wire resistances of suitable voltage which are connected in parallel, a sliding resistance being introduced between the main current and the wire resistances, for the accurate adjustment of the temperature. Very little alteration in the resistance is necessary from day to day, and the temperature does not usually vary as much as  $5^{\circ}\text{C}$  in the course of one day. The air space must be sufficiently large to ensure a uniform temperature round the pentoxide tube which must not come in contact with the heating arrangements.

We should also like to emphasise the importance of correct seasoning of the pentoxide tube. This *must* be done at  $250^{\circ}\text{C}$ , as stated in the original paper. The reason for this appears to be empirical, but we had considerable trouble with one tube which was only seasoned at  $200^{\circ}\text{C}$ , and a number of our results had to be discarded, owing to the consequent irregularity of the reaction. We also find it advisable to heat the tube up to  $250^{\circ}\text{C}$  for  $\frac{1}{2}$ –1 hour whenever it has been out of use for 2 or 3 days—as over the week-end.

The rate at which the air is passed over the pentoxide is also important. The flow must be sufficiently rapid for the completion of the reaction within the time allowed, which is usually 5 minutes, but not so rapid as to allow the escape of iodine through the absorption tube. This latter point can easily be controlled by the addition of a second absorption tube.

(c) *Pulse rate and pulmonary ventilation*. Those who are familiar with the technique of the determination of basal metabolic rate will already have realised the length of time required in some subjects for the respiration to settle down after first beginning to breathe through the valves. We always leave the subject to breathe through the valves to air, for several minutes before turning on the ethyl iodide—the alveolar tube being shut off during this period. The ethyl iodide usually stimulates both the pulse and the respiration slightly, and therefore, although the actual duration of the experiment is 10 minutes, we now take the pulse rate and respiration volume from an average of the last 5 minutes only, as that taken over the whole 10 minutes' period usually gives too high a figure.

## CONCLUSIONS

1 During the last nine months considerably over a 100 determinations of circulation rates have been made by the ethyl iodide method, on 20 different subjects. As a result of these determinations we are convinced that the method gives excellent and consistent results in the majority of normal cases. It is also easy of application.

2 The average figures for circulation rate, stroke volume and stroke index in a group of women are given.

3 The stroke index is the most constant figure in connection with circulation rate, giving an average output for women of 1.7 c.c. per kg. of body weight from the right side of the heart during rest.

Finally, we desire to acknowledge most gratefully the help of Prof. Yandell Henderson, under whose personal supervision the apparatus was set up. We also desire to thank very warmly Lord Riddell, the donor of the research fellowship held by O. R., and Prof. Louise McIlroy, at whose request and in connection with whose department it was awarded. Our grateful thanks are also due to Mr. William Lofts for assistance in constructing the apparatus, and for continued help throughout the work.

Towards the expenses of this research a grant was made by the British Medical Association.

*Note.* Since this paper was written, an article by Dr W. Mobitz (3), giving results of observations on circulation rate by the ethyl iodide method, has appeared. The figure given for the stroke volume in women is 75–100 c.c. which is smaller than in our cases.

Like ourselves, he concludes that consistent results can be obtained, and he also emphasises the necessity for checking the accuracy of the alveolar samples by the Haldane-Priestley method, and for reducing the dead space of the valves.

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THE CHRONAXIE IN TETANY The effect on the chronaxie  
of thyreoparathyreoidectomy, the administration of  
guanidin and of di-methyl guanidin

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INTRODUCTION

SINCE 1874, when Erb(1) first described his classic sign, a hyper-excitability of peripheral nerves to galvanic stimulation has been regarded as a typical manifestation in idiopathic tetany. Recent years have produced much work, notably that of Noël Paton and Findlay(2) and more recently of Frank Stern and Nothmann(3) and György and Vollmer(4) on the experimental production in animals of the symptoms of tetany by parathyreoid removal and by the injection of guanidin and di-methyl guanidin salts. A lowering of the galvanic threshold in a manner strictly similar to that seen in idiopathic tetany is shown to be a constant phenomenon in such conditions experimentally produced.

The present investigation was undertaken to discover what alterations, if any, in excitability were present in tetany produced by parathyreoidectomy and by guanidin and di-methyl guanidin, using as a measure of the excitability the chronaxie instead of the less accurate galvanic reactions.

In an isolated muscle nerve preparation there are various aspects of the process of excitation, conduction and contraction which can be measured quantitatively, but in investigations where the nerve and muscle remain in the body, the only quantities which can be measured readily are those which define the threshold electric stimulus.

The pioneer work on the relations of the minimal current strength to the minimal duration of a stimulating current done by Fick(5), Engelmann(6), Hoorweg(7) and Weiss(8) led to the investigations of Lapicque(9) and Lucas(10) on the form of the strength duration curve for different tissues and then to the theoretical interpretation of this curve by Nernst(11), Lapicque(12) and Hill(13). Lapicque has

introduced two constants, the rheobase and the chronaxie, which are easily measured and which suffice to define the form of the strength duration curve and so to measure the excitability of a tissue with a far greater precision than could be obtained by the ordinary methods of electrical testing used in medicine

The meaning of these two constants has been discussed very fully by Lapicque<sup>(9)</sup>, Bourguignon<sup>(14)</sup>, Strohl<sup>(15)</sup>, Cluzet<sup>(16)</sup> and others, but it will be worth while recalling that they are very simply related to Weiss' empirical equation stating the relation between the strength and the duration of a constant or rectangular current required to excite Weiss' equation (which is not more than a moderate approximation, see Lucas<sup>(10)</sup> and Hill<sup>(13)</sup>) is

$$I = a/t + b \quad (1)$$

or  $Q = a + bt$ , where  $I$  is the current which must flow for a duration  $t$ ,  $Q$  is the quantity of electricity ( $I \times t$ ) which passes, and  $a$  and  $b$  are constants. From equation (1) it follows that if  $t$  is infinite, *i.e.* if the current is allowed to flow for a considerable time, then  $I = b$ . This constant  $b$  Lapicque called the rheobase, and it is measured by finding the current strength needed for excitation when the duration of flow is long (over 1 second). Weiss proposed as his characteristic of an excited tissue the ratio  $a/b$  and a special case of this was taken by Lapicque, and called the "chronaxie".

From our definition,  $\frac{a}{b} = \frac{\text{intensity} \times \text{time}}{\text{intensity}} = \text{a time } t'$  and in equation (1)  $I = a/t + b$ , then  $t' = a/b$  only when  $I = 2b$ .

If we find the least duration  $t = a/b$  corresponding to a strength of current twice as great as the rheobase, then that duration gives us the other constant  $a$  of Weiss' equation. Thus, by determining the rheobase  $b$  and this duration  $t' = a/b$  we can define the whole curve expressed by the equation (1).

Of these two constants, the chronaxie and the rheobase, the former is by far the more significant since it is not affected by changes in tissue resistance, electrode area, etc., but only by changes in the actual time relations of the exciting process. In the present work the chronaxie and the rheobase of various muscles and nerves have been determined before and after thyreoparathyroidectomy and after the injection of guanidin and di-methyl guanidin salts which, it has been suggested, produce symptoms similar to those of post-operative tetany.

*Measurement of the time element.* In previous work on the chronaxie in this country the currents used have been rectangular, *i.e.* increasing

suddenly from zero to the maximal value, remaining at this for a definite time and then decreasing suddenly to zero. Such currents are produced by the ballistic rheonome of Weiss or the pendulum or spring contact breaker designed by Keith Lucas(17). An apparatus of this kind has the advantage of allowing the entire strength duration curve to be mapped out if it should be thought necessary to do so, but for delivering currents of very short durations the mechanical contact breaker is probably not so accurate as the condenser method of Lapicque, and this latter has been used in the present research. When a condenser is discharged through a resistance greater than  $\sqrt{4L/C}$  (where  $L$ , the self-inductance of the system, is negligibly small, and  $C$  is the capacity of the condenser) the current falls exponentially from its maximal value to zero, at a rate depending on the capacity of the condenser and the resistance of the circuit, and in accordance with the equation

$$I_t = I_0 e^{-t/RC} \quad (2)$$

where  $I_t$  is the intensity after a time  $t$ ,  $I_0$  is the initial maximal intensity,  $R$  is the resistance, and  $C$  is the capacity of the condenser. Thus a set of condensers of different capacities will give us a set of currents falling to zero at different rates. To determine the chronaxie by condenser discharges Lapicque proceeds as follows: (1) the rheobase is found by determining the minimal current which will excite if allowed to run continuously (*i.e.* for more than half a second), (2) the voltage required for the rheobase is doubled, and a series of condensers is charged to this value. These condensers are now discharged through the tissue and the smallest capacity which will just excite is determined. From this the chronaxie can be found by multiplying the critical capacity in farads by the resistance in the circuit in ohms and by an empirical constant 37. The value of this constant was found by control experiments with rectangular currents on various tissues (Lapicque(22)).

*Apparatus.* The apparatus used in this investigation was developed for use with invertebrate mammalian and human tissues from that of Lapicque (cf. Fulton (18)) and of Bourguignon (19). The source of current is a 200 volt 10 ampère hour battery of accumulators. The potential reducer is of the type described by Lapicque (20) and is so constructed that by putting over a switch from the "rheobase" to the "chronaxie" side the output is doubled both as regards amperage and voltage. The actual instrument made by Coudou of Paris is a balanced resistance box with 72 ohms on each side and capable of carrying 20 volts. If the voltage applied to the potential reducer was varied by a resistance placed between it and the battery then the voltage and amperage of the output would not be exactly doubled when the switch is changed from "rheobase" to "chronaxie," for although the resistance in the potential reducer would thus be halved that in the battery circuit would remain unchanged. To avoid this error a voltage selector was constructed

of two moving arms each travelling over a series of contact studs mounted in an insulating panel. Wires were led from each of the first ten cells of the battery to their respective studs on the first dial and from each of the remaining nine groups of ten cells to their studs on the second dial. By this system voltages of 2 to 220 in steps of 2 volts could be applied to the potential reducer without having any regulating resistance in the battery circuit. In order to use voltages up to 220 with the potential reducer, compensating resistances each of 72 ohms were non inductively wound from No 32 D C C eureka wire, and so connected that one could be put in series with each of the two box resistances for every 20 volts added from the battery. This produced a constant current output from the box of either 27 or 18 ampère, depending on the position of the switch, so the rheobase could be measured in volts instead of in milliamperes.

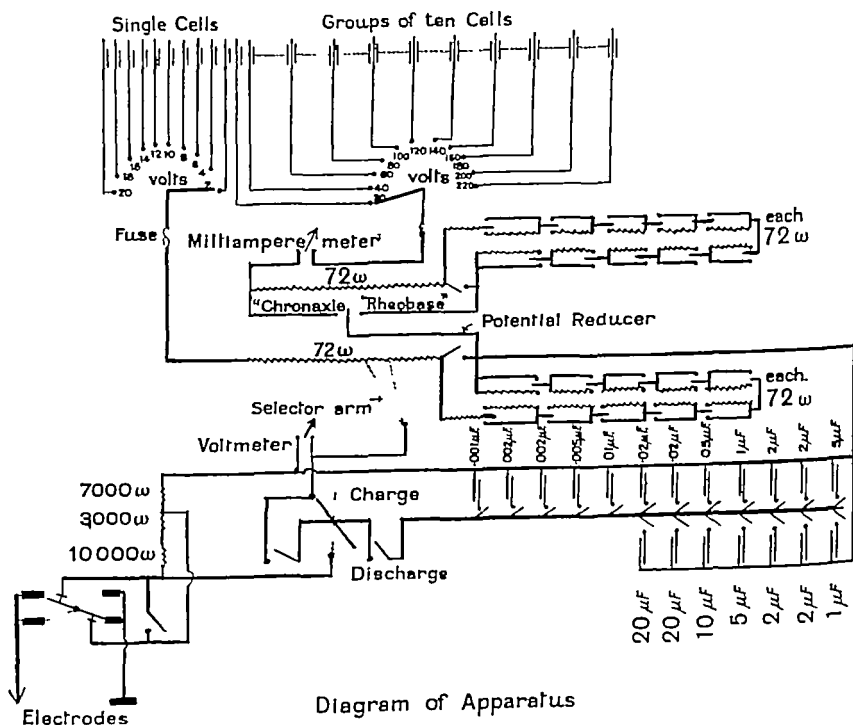


Diagram of Apparatus

A group of standard mica condensers connected in parallel was obtained from the Dubilier Condenser Co of the following values 5, 2, 2, 1, 0.5, 0.2, 0.2, 0.1, 0.05, 0.02, 0.02, 0.01  $\mu F$ . This range of values is sufficient for all frog work and most of the mammalian and human investigation, but in some cases higher values are needed and these were obtained by building up 1  $\mu F$  and 2  $\mu F$  units of the paraffin paper type into groups giving 20, 20, 10, 5, 2, 2, 1  $\mu F$ . All these units are connected in parallel and each value has a separate key.

The resistances used in the shunt circuits, suggested by Lapicque (18) and Bourguignon (21), to minimise the effect of the altering resistance of the subjects and of the electrodes are wound non inductively of manganin wire. The electrodes used for percutaneous stimulation are of two types (a) two disc electrodes of sterling silver 2 mm thick,

one 4 cm. and the other 1 cm. in diameter, with a silver connecting rod 7 cm. long, soldered to the centre of one face with silver solder. These discs were sunk in flat vulcanite cups and covered with asbestos and gauze as described by Bourguignon (22). (b) A small electrode for stimulating small motor points was made from glass tubing 9 cm. long and 4 mm. bore drawn to a fine point and covered on the outside with rubber tubing to render it opaque. A coil of fine silver wire projects into this tube through a cork at the larger end and the point of the tube is plugged with asbestos and gauze.

The surface of the discs *A* and of the wire in *B* was covered with silver chloride by immersing them in 4% NaCl solution and passing for an hour a current of 20 m. a. through them as anode, using a platinum wire as the cathode. During use the electrodes were kept saturated with 4% NaCl and the glass tube was kept filled with the same solution. The large 4 cm. disc was always used as the indifferent electrode and only cathodal stimulations were observed in making any measurement of the rheobase or of the chronaxie. During an observation the current was reversed in direction after each stimulation, to break down the polarisation of the tissues and to prevent the destruction of the coat of silver chloride on the cathode.

### EXPERIMENTAL

Different muscles of the hind limb were used during the investigation in an attempt to find the one which gave the most constant results and in which the reactions were the most easily and quickly measured. The most suitable muscle was found to be a deep flexor and next in order an extensor. The objections to the gastrocnemius muscle are that it is crossed by fibres of the vastus externus whose contractions can be confused with those of the underlying gastrocnemius and that the peculiar end distribution of its motor nerves leads to conflicting results. The stimulation of a nerve trunk is a much more difficult proceeding than the stimulation of a muscle through its motor point and to obtain reliable results requires great care and considerable practice with a suitable electrode. The sciatic nerve was investigated in animals 1, 2, 3, 6, 7 and 8 and the common peroneal in animals 4 and 5.

Bourguignon (23) brings forward convincing evidence that in the normal animal percutaneous stimulation of a muscle through its motor point or through a nerve trunk is a stimulation of a nerve and that to obtain direct muscular excitation necessitates previous denervation of the selected part either by nerve section or curarisation.

All the measurements in this series were made on cats and the following procedure was followed in each case. The fur was shaved from the external surface of the thigh and lower leg of one side, and on the same side from an area on the thorax slightly larger than that of the large indifferent electrode. This procedure is not absolutely necessary as all alterations in the body resistance are allowed for in the shunt circuit of the electrodes but it reduces the resistance and allows a much lower



voltage to be used as the rheobase, and very materially assists in the accurate observation of the minimal muscular contraction

No anæsthetics of any kind were used during these investigations, as the animals could always be controlled without them. For the first few days if the cat would not lie still while the readings were being taken it was placed with the one hind leg protruding, in a castration bag in which a hole had been cut for the large electrode. There was no possibility of any compression causing alteration of the respiration or of the circulation, and it was always found that the bag could be dispensed with after a few days. Three persons were necessary to make an observation. The first controlled the animal and held the large electrode in position, the second adjusted the stimulating current, and the third, using the small active pole, observed the reaction. The apparatus was so arranged that the actual observer could not see the readings on the dials and had to ask for "more" or "less" without knowing the actual values he was using. This precaution was absolutely necessary to prevent preconceived ideas influencing the results.

## RESULTS

### A *In the normal animal*

(1) *The daily physiological variation* In each animal the rheobase and the chronaxie were measured for several days before any experimental treatment was carried out, and in addition to this two cats were kept as normal controls and their reactions were measured daily for a period in one case of over four weeks.

The daily variation in the chronaxie in the deep flexor of cat 13 and in the extensor of cat 10 are shown in Table I, and Figs 1, 2 and 3 show the readings for the sciatic nerve, the deep flexor and the semi-tendinosus in cat 8 which may be taken as typical examples of the whole group.

TABLE I. Daily physiological variations.

<i>Deep flexor Cat 13</i>			<i>Extensor Cat 10</i>		
Date	Rheobase (volts)	Chronaxie (1/1000 sec)	Date	Rheobase (volts)	Chronaxie (1/1000 sec)
5 m. 25	42	11	13 1 25	35	26
	40	15	14 1 25	18	37
	20	15	15 1 25	18	33
	24	16		20	33
	15	11	16 1 25	40	33
7 m. 25	22	11		18	26
	16	11		22	55
	20	07	17 1 25	35	44
	20	15		28	37
9 m. 25	18	15	18 1 25	53	26

The extent of these variations is not great but the rheobase which corresponds to the  $\kappa c c$  of electrical reactions shows a much greater range of values than the chronaxie

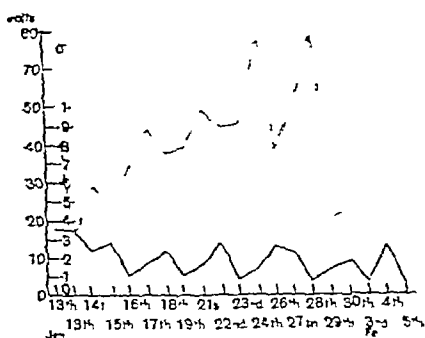


Fig 1. Cat 8. Sciatic nerve (normal)  
Abscissa gives separate observations.  
— Chronaxie in  $\sigma = 1/1000$  sec.  
- - Rheobase in volts.

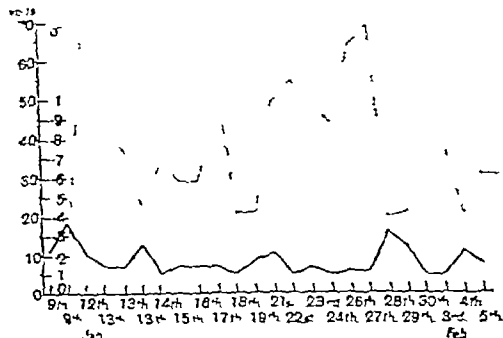


Fig 2. Cat 8. Deep flexors (normal)  
Abscissa gives separate observations.  
— Chronaxie in  $\sigma = 1/1000$  sec.  
- - Rheobase in volts.

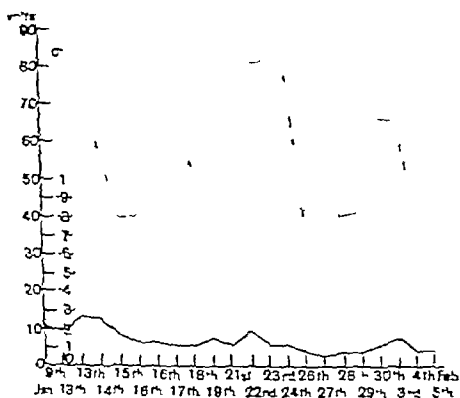


Fig 3 Cat 8 Semitendinosus (normal)  
Abscissa gives separate observations.  
— Chronaxie in  $\sigma$  - - Rheobase in volts.

(2) *Anæsthetics (ether and chloroform)* The following experiment shows how profoundly the rheobase and the chronaxie are modified by anæsthetics

Normal unanæsthetised

Normal during complete ether anæsthesia

Normal 15 mins after the anæsthetic was stopped

Cat 1 10th Sept 1924.

Deep flexor muscle of right hind leg Rheobase 51 volts.

Chronaxie 38 $\sigma$

Rheobase higher than 160 volts Chronaxie could not be measured since the rheobase was so high.

Pheobase 104 volts Chronaxie 148 $\sigma$

The rheobase and the chronaxie gradually fall and reach the normal level in from 3 to 6 hours after the anæsthetic is removed. From these results it is obviously not permissible to use an anæsthetic such as ether or chloroform to control an animal during the taking of observations, and to avoid the phase of decreased excitability after anaesthesia no readings were taken in any of our post-operative conditions, described later, until at least 12 hours had elapsed.

### B *In tetania parathyreopriva*

The operation performed in all our cases was that of complete thyreoparathyroidectomy and no attempt was made to achieve pure parathyroidectomy because of the wide variation in position of the internal parathyroids and of the frequent presence of accessory bodies. The presence or absence of the thyreoid has nothing to do with the onset of tetany and the symptoms of removal of the thyreoid apart from the parathyroids develops late and therefore in no way interferes with the manifestations of tetania parathyreopriva (see Noël Paton and Findlay (24) for further details on this point, and also for a complete picture of the symptoms of the usual post-operative condition).

*The chronaxie* There is always an appreciable rise in the value of the chronaxie after this operation, which may in some cases occur at the onset of symptoms and in others near the end, but which does not seem to correspond directly with the severity of the symptoms. The rise in the chronaxie is more noticeable in the case of a nerve, such as the sciatic, as recorded in Table II, than in a muscle. Fig 5 shows for the deep flexor in cat 10, a marked initial rise in value, followed by a gradual fall and then by a second rise just before death, and Figs 4 and 6 show the height to which the chronaxie of a muscle may rise in this condition.

The rheobase is generally reduced after operation but it does not show any definite relation to the value of the chronaxie or to the severity of the symptoms.

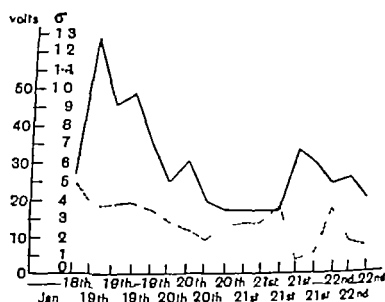
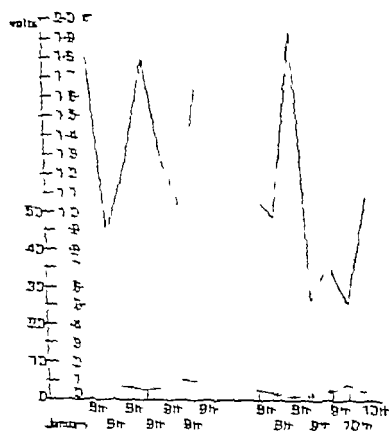


Fig 5 Cat 10 Deep flexors After thyreoparathyroidectomy on January the 18th.  
Abscissa gives separate observations.  
— Chronaxie in  $\sigma = 1/1000$  sec  
Rheobase in volts.

TABLE II. After thyro-parathyroidectomy

Smith notes Cat 5.

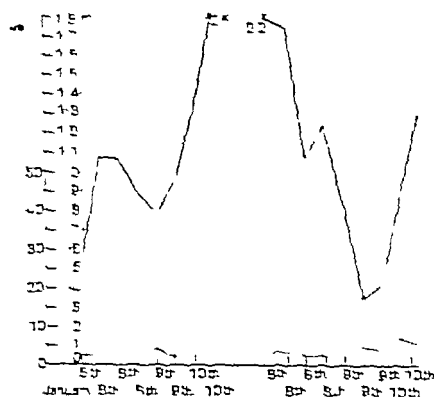
Date	Rheobase (volts)	Chronaxie ( $c=1/1000$ sec.)	Date	Rheobase (volts)	Chronaxie ( $c=1/1000$ sec.)
13.1.25	56	18 c	27.1.25	12	33 c
14.1.25	45	44 "		12	37 "
15.1.25	12	26 "		10	* 18 "
	18	27 "		10	34 "
16.1.25	49	28 "		6	55 "
	20	33 "	21.1.25	5	15 "
	15	33 "		7	15 "
17.1.25	10	37 "		21	29 "
	12	40 "		15	27 "
18.1.25	24	57 "		14	23 "
18.1.25	17 removed			8	21 "
19.1.25	28	60 "	22.1.25	17	14 "
	25	125 "		11	10 "
	21	93 "		19	16 "
	30	120 "			
	16	88 "			
	20	129 "			



Cat 6

Cat 7

Fig. 4.



Cat 6

Cat 7

Fig. 6.

Fig. 4. Deep flexors. First reading taken in both cases 24 hours after thyro-parathyroidectomy

Fig. 6. Semitendinosus. First reading taken in both cases 24 hours after thyro-parathyroidectomy

Abscissa gives separate observations.

— Chronaxie in c. - - - Rheobase in volts.

The rheobase and the chronaxie gradually fall and reach the normal level in from 3 to 6 hours after the anæsthetic is removed. From these results it is obviously not permissible to use an anæsthetic such as ether or chloroform to control an animal during the taking of observations, and to avoid the phase of decreased excitability after anæsthesia no readings were taken in any of our post-operative conditions, described later, until at least 12 hours had elapsed.

### B *In tetania parathyreopriva*

The operation performed in all our cases was that of complete thyreoparathyroidectomy and no attempt was made to achieve pure parathyroidectomy because of the wide variation in position of the internal parathyroids and of the frequent presence of accessory bodies. The presence or absence of the thyreoid has nothing to do with the onset of tetany and the symptoms of removal of the thyreoid apart from the parathyroids develops late and therefore in no way interferes with the manifestations of tetania parathyreopriva (see Noel Paton and Findlay<sup>(24)</sup> for further details on this point, and also for a complete picture of the symptoms of the usual post-operative condition).

*The chronaxie* There is always an appreciable rise in the value of the chronaxie after this operation, which may in some cases occur at the onset of symptoms and in others near the end, but which does not seem to correspond directly with the severity of the symptoms. The rise in the chronaxie is more noticeable in the case of a nerve, such as the sciatic, as recorded in Table II, than in a muscle. Fig 5 shows for the deep flexor in cat 10, a marked initial rise in value, followed by a gradual fall and then by a second rise just before death, and Figs 4 and 6 show the height to which the chronaxie of a muscle may rise in this condition.

The rheobase is generally reduced after operation but it does not show any definite relation to the value of the chronaxie or to the severity of the symptoms.

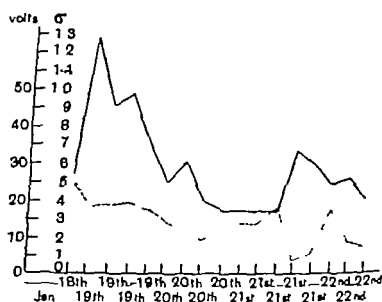


Fig 5 Cat 10 Deep flexors. After thyreoparathyroidectomy on January the 18th.

Abscissa gives separate observations.

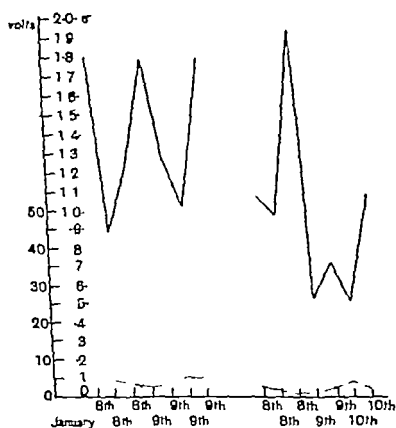
— Chronaxie in  $\sigma = 1/1000$  sec.

- - - Rheobase in volts

TABLE II After thyreoparathyroidectomy

Sciatic nerve Cat 5

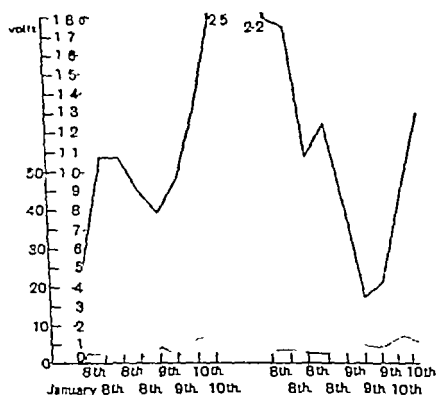
Date	Rheobase (volts)	Chronaxie ( $\sigma=1/1000$ sec.)	Date	Rheobase (volts)	Chronaxie ( $\sigma=1/1000$ sec.)
13 i. 25	56	13 $\sigma$	20 i 25	12	33 $\sigma$
14 i. 25	45	44,,		12	37,,
15 i. 25	12	26,,		10	518,,
	18	37,,		10	54,,
16 i. 25	40	26,,		6	55,,
	20	33,,	21 i 25	5	15,,
	15	33,,		7	15,,
17 i 25	10	37,,		21	03,,
	12	40,,		15	26,,
18 i 25	24	37,,		14	22,,
18. i. 25	T.P. ectomised			9	277,,
19 i. 25	28	60,,	22. i 25	17	148,,
	26	185,,		11	107,,
	21	93,,		19	167,,
	30	120,,			
	16	88,,			
	20	129,,			



Cat 6

Cat 7

Fig 4.



Cat 6

Cat 7

Fig 6

Fig 4. Deep flexors. First reading taken in both cases 24 hours after thyreo parathyroidectomy

Fig 6 Semitendinosus. First reading taken in both cases 24 hours after thyreo parathyroidectomy

Absoissa gives separate observations.

— Chronaxie in  $\sigma$  - Rheobase in volts

C *After the injection of guanidin chloride and di-methyl  
guanidin chloride*

The records of a typical case of a 1100 grm cat treated with guanidin show that on the first day a subcutaneous injection in normal saline was given of 0.6 grm, on the second day 1 grm, on the fifth day 1 grm, on the sixth day 2 grm and that on the seventh day the animal was killed with ether and chloroform anaesthesia. The signs which appeared after these injections were increased salivation, vomiting, diarrhoea, stertor and photophobia, with irregular tremor and twitchings of the whole body and impairment of balance so that the animal walked with a staggering gait. Di-methyl guanidin being a more toxic salt was given in two doses, each of 0.3 grm, for each kilo of body weight, on two successive days, and the animal was killed at the end of the third day. This produced very marked signs which on the whole were similar to those caused by guanidin except that diarrhoea was absent and that the respiration rate was increased from the normal 30–40/min to 80–100/min, a laryngeal spasm appeared and the animal almost continually rubbed its nose with its front paws. In one case there also developed a marked internal strabismus accompanied with a strong retraction of the head as in meningitis. Watershake, so typical a sign in tetania parathyreo-priva, was very seldom seen in guanidin intoxication and never appeared at all in those animals in which di-methyl guanidin was injected.

*The chronaxie* There is a very slight decrease from the normal daily range after the injection of guanidin which may be due to the action of this drug, but the administration of di-methyl guanidin, even in sufficient doses to produce very marked symptoms, leaves the chronaxie unaltered.

*The rheobase* After the injection of either guanidin or di-methyl guanidin this does not show that reduction in value below the normal

TABLE III. After the injection of guanidin chloride

Date	Deep flexors Cat 8	
	Rheobase (volts)	Chronaxie ( $\sigma = 1/1000$ sec)
	Av before injection	17 $\sigma$
5 II 25	30	13,,
6 II 25	30	26,,
	36	22,,
7 II 25	35	15,,
9 II 25	31	07,,
	39	15,,
10 II 25	40	26,,
	30	20
	30	37,,
	30	15

0.6 grm guan. cl injected  
1 grm guan. cl injected

1 grm guan. cl. injected

2 grm. guan. cl. injected

level seen in post-operative tetany but remains within the limits of the normal daily range

Tables III and IV and Figs 7 and 8 give actual readings and diagrams of the results after injection in four cases

TABLE IV After the injection of di methyl guanidin chloride

Date	Rheobase (volts)	Chronaxie ( $c \approx 1/1000$ sec.)	
	Av before injection	24c	
2 m. 25	14	22	-05 gm. di m. g. cl. injected
	15	28	
	22	19	
	25	20	
	25	20	
3 m. 25	18	15	0.5 grm. di m g cl. injected
	15	11	
	30	26	
	30	19	
	10	19	
	14	19	
4 m. 25	21	-07	
	23	11	
	31	26	
	28	19	
	20	11	
	15	-09	

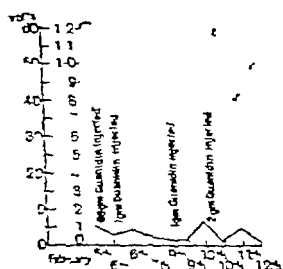


Fig 7 Cat 8. Semutendmosus.  
After guanidin injection

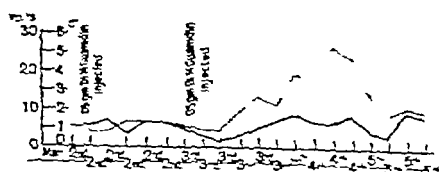


Fig 8 Cat 12. Deep flexors. After  
di methyl guanidin injection.

Abscissa gives separate observations  
 — Chronaxie in  $c$     - - - Rheobase in volts.

## DISCUSSION

The results put forward here, that there is an increase in the chronaxie or excitation time in the tetany of parathyroidectomy is rather the reverse of what previous work would lead one to expect. It must be remembered, however, that it is the rheobase which corresponds to the  $KCC$



of electrical reactions and that that does show a definite reduction, and that the  $\kappa$  o c of Erb's sign has no counterpart in either the rheobase or the chronaxie which are both kathodal closing contractions. The rheobase depends for its value on the electrical resistance between the two electrodes as well as on the state of excitability of the stimulated motor point and therefore the decrease in the rheobase in tetany might imply no more than a decrease in the electrical resistance of the skin. It is difficult to see, however, how such an alteration of resistance could lead to the increased excitability to mechanical stimuli shown in Chvostek and Trousseau's signs, unless it is that these reactions are reflexes through the proprioceptive nerves as Dittler and Freudenberg(25) have suggested. The chronaxie, on the other hand, does not depend on the interelectrode resistance but on the time relations of the excitation process, and when the chronaxie is increased, as in parathyroidectomy, it means that the intensity of twice the rheobase must act on the tissues for a longer period than is normal before the excitation process is set going. Results similar to ours have been obtained by Bourguignon and Haldane(26) in the tetany produced by forced breathing. They found that during tetany the chronaxie was increased both on the nerve and on the muscle and that the value, although never greater than six times the normal on the muscle, was usually ten times greater on the nerve and in some cases as much as twenty to thirty times. It is interesting to note also that the results of Noel Paton and Findlay(27), Frank Stern and Nothmann(3) and György and Vollmer(4), which in some cases at least show a definite lowering of the  $\kappa$  o c after guanidin and di-methyl guanidin injection, were not confirmed in our results and also that there was no increase in the chronaxie in these conditions as in parathyroidectomy. Although a consideration of the general symptoms in these three conditions shows at once their similarity, it seems true that so far as concerns the electrical signs of these conditions they are not comparable.

#### CONCLUSIONS

- 1 The chronaxie shows much smaller daily variations in value than the rheobase

- 2 The value of the chronaxie and of the rheobase may be completely altered by the anæsthetising by chloroform or ether of the animal investigated, so that readings taken under such circumstances cannot be accepted as an index of the state of excitability of the motor nerves or of the muscles which they innervate

3 Thyreo-parathyreoidectomy very markedly increases the value of the chronaxie and to a lesser degree decreases the value of the rheobase

4 These alterations in value have no direct and evident relation to the severity of the accompanying symptoms

5 Guanidin chloride or di-methyl guanidin chloride as administered by us has no effect on the value of the chronaxie or of the rheobase

It is a great pleasure to us to acknowledge our indebtedness to Prof D Noël Paton for his constant sympathy and advice throughout the course of these investigations

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# EFFECT UPON PLATELETS AND ON BLOOD COAGULATION OF INJECTING FOREIGN PARTICLES INTO THE BLOOD STREAM

BY JOHN TAIT AND A R ELVIDGE<sup>1</sup>

(From the Department of Physiology and Experimental Medicine,  
McGill University, Montreal, Canada)

It was pointed out by Tait<sup>(14)</sup> that many blood cells on contact with an area of non-greasy foreign material are, by capillary action, pulled progressively outwards over the foreign surface, like a drop of water on a stone. To such cells, which include mammalian platelets, many of the spindle-cells of amphibians and fishes, certain special cells of the blood of *Astacus* and of some other crustacea, and various corpuscles of mollusca, arthropoda and annulata, he applied the name *thigmocytes*. It is characteristic of thigmocytes that they are not distorted by contact with an oily or greasy surface, on the other hand, they are highly phagocytic towards minute particles of non-greasy matter.

In bloods that possess the property of true jelly coagulation, e.g. vertebrate blood or the blood of certain special crustacea (Heim<sup>(7)</sup>, Tait<sup>(13)</sup>), the thigmocytes act as a source of thrombin. When, by capillary action, they have once been stretched beyond a certain limit, a sudden cytolytic disintegration occurs. Tait and Gunn<sup>(17)</sup> were able to show that cytolysis of the hyaline thigmocytes of *Astacus*, which occurs some 15-20 minutes after the blood is shed, is immediately followed by a coagulation of the surrounding plasma. Tait and Burke<sup>(15)</sup> demonstrated that mammalian platelets, having once attained a certain degree of expansion on a glass slide, suffer an equally abrupt disintegration and, like the hyaline thigmocytes of *Astacus*, discharge globules of thrombin into the plasma. That the stimulus which leads to cytolysis of thigmocytes is purely physical was proved by Tait and Green<sup>(16)</sup>.

The fact that thigmocytes, without detriment to their stability, may engulf small particles of a given water-wettable material, while on a larger slab of the same material they suffer disaster in their efforts at phagocytosis, indicates that simple contact with foreign matter is not

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sufficient to determine disintegration of the cells. The structural collapse of these elements would appear to result from over-stretching. In the blood stream of some animals there are, however, cells of even more labile constitution, *e g* the explosive corpuscles of *Astacus* (Hardy(6), Tait and Gunn(17)) and of *Ligia* (Tait(12)), and possibly some of the cells of amphibians (Meves(9)). These appear to be still more sensitive to contact with water-wettable foreign matter. So delicate is their structural organisation that any little surface disturbance of a capillary kind leads forthwith to disintegration. In the nature of things such cells are not phagocytic (Tait and Gunn(17)). At the same time they, too, yield thrombin to the blood (cf Tait(12), Tait and Gunn(17) and Tait and Green(16)).

In view of the thigmocytic reaction of mammalian platelets on a slab of water-wettable material and their corresponding power to ingest very minute particles without apparently suffering cytolysis, we arranged to inject into the blood stream minute particles of insoluble material which had been carefully graded according to size. It was possible, for example, that injection of particles of a size so small that they could be comfortably accommodated within the platelets might lead to no conspicuous reaction, whereas injection of particles of a diameter as large as red blood corpuscles might induce intravascular coagulation. It was eventually possible from the figure in Tait and Burke's(15) paper to estimate approximately the maximal area of expansion of both large and small platelets, which is some 100 sq  $\mu$  for the large and some 40 sq  $\mu$  for the small. These data were not, however, available when we began our experiments.

The only experiments known to us in which insoluble particles were deliberately added to blood with the object of inducing coagulation, are those of Rettger(10), who showed that "dust particles, loose sweepings, or linty shreds" added to centrifuged terrapin plasma induce a prompt coagulation. From the fact that both dust and long irrigated tissue extract produce similar coagulation in this reptilian plasma, Rettger was inclined to conclude, in opposition to the unvarying testimony of other workers, that tissue extract does not possess any peculiar or specific coagulating virtue. No authors appear to have investigated the possible coagulant effect of introducing foreign particles into the circulating current. At the same time the investigation is a necessary one, for such odd fragments of knowledge as we already possess in respect to platelets indicate high functional rank for these elements in a variety of processes not always suspected of having im-

mediate interrelation. In a succeeding paper (by J T) it will be shown anew how vital is the part they play in arresting hæmorrhage from a wound. They, and they alone, would appear to be involved in hæmophilia. In most thromboses they are the important agents. They play an important part in certain immunity reactions (Govaerts(5)). They are undoubtedly phagocytic, and, as phagocytes or as adhesive "grips" probably play a part in anchorage and disposal of blood-carried micro-organisms. Their special relation to the spleen has been shown by the work of Bedson(1). The all but universal occurrence of such elements, at least in animals possessed of a circulatory system<sup>1</sup>, is a further indication of their general importance.

The platelets or hyaline thrombocytes of mammals are peculiar in at least two respects, in point of size and in apparent absence of a nucleus. Their minute size may be an adaptation to the diminutive proportions of invading organisms. Their non-nucleate or sub-nucleate condition, implying absence or defect of constructive metabolism, might suggest in their case a special adaptation as simple adhesive "grips" when anchorage of foreign circulating material is concerned.

#### TECHNICAL DETAILS

*Preparation of quartz suspensions* The material chosen for preparation of the particles was fused quartz. (Were we to repeat the experiments we should use crystalline quartz, because of the possibility of subsequently detecting the particles in the tissues with a Nicol prism.) Transparent fragments, obtained from the Thermal Syndicate, Ltd., Wallsend-on-Tyne, England, were first subjected to a process of chemical purification for the purpose of removing grease and adherent soluble material. Between hard steel faces the dried pieces were broken into smaller fragments, which were then pounded to dust in a diamond mortar. The dust was now ground for 48 hours in a mechanically driven agate mortar. During the first 12 hours it remains heavy and sandy, later it commences to fluff up and becomes progressively lighter and more mobile. The ultimate fine product which, like "light magnesia," slides from side to side in the containing vessel, was stored in flasks of hard glass which had been chemically cleaned. Throughout these manipulations the quartz is never touched with the fingers, all precautions being taken to furnish clean surfaces of fracture or of attrition.

By levigation in high-grade conductivity water the particles were now assorted into graded sizes. The water of suspension had been sub-

<sup>1</sup> Hyaline thrombocytes do not seem to exist in the body fluid of Echinodermata.

jected to five distillations, twice through glass, twice through special stills of "vitreosil" ware in which no cork or rubber joins were used. Before the fifth (and final) distillation a small quantity of potassium permanganate and sodium hydroxide was added to the distilling flask, in accordance with the recommendation of Kraus and Dexter<sup>(8)</sup>. The object of this repeated distillation was to obtain water as free from electrolytes as possible.

The distilling flasks, provided with special necks, which rendered corks unnecessary, were of pyrex glass. The vessels for holding the ultimate conductivity water or quartz suspensions as the case might be, were of vitreosil or of clear fused quartz. All our grades of suspension showed Brownian movement, feeble, in the case of the coarse, and active in the case of the fine suspensions. The complete absence of agglutination and the continued presence of this Brownian movement over a period of days on end, sufficiently attested the absence of any contamination with electrolytes. After settling of a suspension complete dispersion at once occurred on shaking.

According to Stokes' law the speed of sedimentation,  $V$ , is directly proportional to  $F$ , the force of gravity, and inversely proportional to  $\eta$ , the viscosity of the dispersion medium, as well as to  $r$ , the radius of the particle, in accordance with the formula

$$V = F/6\pi\eta r$$

Good agreement was found to obtain between the formula and the actual rate of sedimentation of the particles. At 18° C particles just a little smaller than the red blood cells of the rabbit (6.5–7  $\mu$  in diameter) were, after 1½ hours, still held in the top inch of the fluid. Particles measuring from 2.5 to 3  $\mu$  in diameter remained in the top inch after 3 hours' settling. Particles less than 1.5  $\mu$  remained in the top inch after 24 hours' settling. Naturally it was necessary to levigate repeatedly in order to obtain uniform grades of suspension. Direct microscopic examination served as a complete check on uniformity. In what follows we shall designate particles 6–7  $\mu$  in diameter, with a calculated surface of some 113–154 sq  $\mu$ , as "coarse quartz", particles from 3 to 3.5  $\mu$ , with a surface of some 13–39 sq  $\mu$ , as "medium quartz", and particles less than 1.5  $\mu$ , with a calculated surface of 7 sq  $\mu$  and under, as "fine quartz".

The point of saturation of colloidal suspensions seems to be variable, the highest determination hitherto recorded being that of Zsigmondy<sup>(18)</sup>, who obtained a permanent suspension containing 0.12 p.c. of gold sol.

On the other hand, Bredig<sup>(2)</sup> gave 0.014 p.c. as the maximum figure for a gold sol. On further concentration gold sols change colour, the change of colour indicating that agglutination has occurred. In our experiments, curiously enough, we could obtain no indication that mere high concentration tends towards agglutination. It was easily possible to hold 6 p.c. of the coarse suspension in complete dispersion for a time at least in the conductivity water. A concentration of 2.03 p.c. medium quartz without any agglutination was obtained. The fact that any single highly concentrated sample, after settling, could forthwith be re-dispersed by shaking, also proves that no effective agglutination ever occurred. The ease with which high concentration can be obtained speaks for a low surface energy. One might be led to suppose that the suspended quartz particles are surrounded by a hydrolytic layer.

Besides particles of quartz we used particles of Indian ink (already in finest suspension when purchased as "Pelican" brand), of barium sulphate and of carmine. After prolonged grinding in the agate mortar the last two substances fluff up in the same way as quartz. These other materials were used chiefly to check the results, for very finely divided quartz is not wholly insoluble. If an effect due to an injection of a quartz suspension can be reproduced when any kind of finely divided particles is substituted for the quartz, the conclusion is that simple particulate matter and not the presence of silicate in solution is responsible for the reaction.

*Means of securing isotonicity* In some early experiments in which the salt constituents of buffered Ringer's fluid were added to the pure quartz suspension just before use, it was found that agglutination occurs so quickly as to block the needle of the hypodermic syringe, while the injected animals die at once of a coagulation which extends along the vein selected for injection to the right heart and thence along the pulmonary artery. A control experiment in which the individual salt constituents of buffered Ringer's fluid were separately added in their appropriate amounts to supplies of the quartz suspension, showed that the bivalent Ca-ion is by far the most effective agglutinant. An efficacious but somewhat tedious method of avoiding agglutination on addition of the salt is to take advantage of the protective action of serum proteins, thus. Equal quantities of fresh serum and of suspension are first mixed together, whereupon the appropriate quantities of NaCl, KCl, CaCl<sub>2</sub> and NaHCO<sub>3</sub> for the amount of quartz suspensions are stirred in. No agglutination occurs, the mixture being more than ever stable and slow of sedimentation. As serum, however, contains thrombin, which is



objectionable, the method we ultimately adopted and consistently used was to add 6 p.c. of pure glucose to the suspension just before injection into mammals and 4 p.c. before injection into frogs. By this means the fluid was rendered isotonic without the necessity of adding electrolytes, the presence of the glucose does not appear in any way to modify the result so far as the platelets are concerned.

*Animals used and sites of injection* In some early experiments carried out on guinea-pigs the quartz was injected into the femoral vein. Owing to the difficulty of carrying out repeated injections, which necessitate anaesthesia and aseptic technique, guinea-pigs were discarded in favour of rabbits, into which the material may be easily introduced by way of the ear vein. A number of experiments were carried out on anaesthetised cats. It soon appeared that the needle-point may be introduced directly into the external saphenous vein of the cat without previous incision of the skin. By this device time is saved, while the anaesthetised animal runs less risk of septic infection and has, moreover, a shorter anaesthesia. In a small number of cases frogs were used, the injection being made directly into the cavity of the ventricle.

## RESULTS

### (a) Intravascular admixture

*Coarse quartz suspensions* Our earliest experiments, when no particular precautions were taken to protect against agglutination of the particles, seemed to indicate that coarse suspensions may cause coagulation, whereas fine suspensions do not. On repeating the work, however, with particles suspended in dextrose solution, we failed to obtain coagulation by injection of coarse quartz. Table I gives a record of 12 experiments, ten on rabbits and two on guinea-pigs, in which amounts

TABLE I (Coarse quartz suspensions)

	Dose in grm per kg	c.c. of fluid injected	Percentage of quartz in suspension	Remarks
Rabbit A	0.043	10	1.07	—
" B	0.13	9	2.36	—
" C	0.11	9	2.36	—
" D	0.12	10	2.36	—
" E	0.15	10	2.56	Death in 1 day
" F	0.19	11	2.56	—
" G	0.23	36	1.07	—
" H	0.25	40	1.87	Killed for examination after 15 minutes
" I	0.50	25	4.05	Death in 3½ hours
" J	0.50	25	4.05	Death in 8 hours
Guinea pig A	0.062	9	0.69	—
" B	0.23	10	2.36	Death in 17 hours

of quartz varying from 0.04 to 0.5 gm. per kg. were injected. Even when the animals died as a result of the injection, e.g. rabbits I and J, the blood was found to be perfectly fluid. So, too, in rabbit H, which was killed 15 minutes after injection, no sign of thrombosis was discoverable.

*Medium and fine suspensions* Experiments similar to those recorded in Table I were carried out on many occasions with medium and fine suspensions (cf. Elvidge(4)). In no case did intravascular coagulation result.

### (b) Extravascular admixture

*Addition of quartz to drawn blood* In the preceding experiments the foreign particles were added to the circulating blood. We know, however, that direct addition, even of thrombin, to circulating blood fails to clot it, which means that in the body the blood is to some degree protected against influences which would otherwise induce coagulation. Consequently it was necessary to know whether admixture with quartz particles initiates or accelerates the coagulation of drawn blood. The blood used in such experiments must of course be shielded from any contact with other water-wettable foreign matter or with recently intermingled tissue-juice. Frog's blood, which is easy to handle, was first used. As it is difficult, if not impossible, to keep mammalian blood permanently fluid, we employed instead (1) cooled mammalian plasma, which has a full content of thrombocytes, and (2) negative phase mammalian plasma (prepared by repeated slow injections of dilute tissue-juice), which, owing to its low content in thrombocytes, clots very slowly on a paraffined surface (cf. Burke and Tait(3)).

From one and the same frog four samples of blood, consisting of two drops each, were removed and preserved in a moist chamber on successive paraffined watch-glasses after the method described by Tait and Green(16). Two of the samples (1 and 3) were kept as controls. To each of the other two samples (2 and 4) were added two drops of coarse quartz dextrose suspension. Each watch-glass in turn was thereupon agitated for a few seconds so as to roll the blood about. The result was as follows:

- No. 1. No coagulation in 24 hours.
- No. 2. Solid coagulation in 6 minutes.
- No. 3. No coagulation in 24 hours.
- No. 4. Solid coagulation in 5½ minutes.

These experiments were repeated on several occasions with similar findings. A pinch of dry (unlevigated) quartz particles dropped into

fluid frog's blood on paraffin also induces prompt coagulation. It is therefore plain that the drawn blood of the frog is induced to coagulate on contact with coarse quartz suspension.

By injecting directly into the ventricle we carried out the converse experiment of mingling coarse quartz with the circulating blood of the frog. In order to examine for thrombosis, which does not here produce sudden death as it does in the mammal, we sometimes watched the mesentery, at other times we displaced the blood by perfusion of the vessels. The results were not so uniformly negative as when quartz suspension is introduced into the circulatory current of mammals, and we did on some occasions produce thrombosis. At the same time both the dose administered and the rate of injection were relatively greater than in the corresponding mammalian experiments.

The effect of adding *fine* quartz suspension to drawn frog's blood is of special interest. Provided a very small quantity of the suspension is added, coagulation does not occur. If the quantity is increased, clotting results. The following experiments illustrate this fact.

- (1) 3 drops blood + a minute drop of fine quartz suspension  
no coagulation in 2 hours and 40 minutes  
Large drop of fine quartz now added  
complete coagulation in  $6\frac{1}{2}$  minutes
- (2) 3 drops blood + a minute drop of fine suspension  
no coagulation in 1 hour and 47 minutes.  
Large drop of fine quartz now added  
complete coagulation in  $5\frac{1}{2}$  minutes.
- (3) 3 drops blood + a large drop of fine suspension  
complete coagulation in 11 minutes.
- (4) 4 drops blood + a large drop of fine suspension  
partial coagulation in  $3\frac{1}{2}$  minutes  
complete coagulation in 30 minutes

The simplest interpretation to put on these experiments is that the spindle-cells rupture only when they have engorged an excess of fine particles.

The experiments on the drawn blood of mammals were just as decisive in establishing coagulant action as those on the drawn blood of the frog. We shall first deal with the cooled plasma. On three occasions at different dates three samples each of cooled mammalian plasma, procured as described by Tait and Burke(15), were placed on paraffined watch-glasses at room temperature. One sample in each case acted as a control. To each of the other two, cooled medium quartz suspension was added. The results were as follows.

*Exp A*

- 1 Plasma control clotted in 20 minutes.
- 2 Plasma + medium quartz clotted in 7½ minutes.
- 3 Plasma + medium quartz clotted in 5 minutes.

*Exp B*

1. Plasma control no coagulation in 1 hour
- 2 Plasma + medium quartz clotted in 12 minutes
- 3 Plasma + medium quartz clotted in 10 minutes

*Exp C*

- 1 Plasma control clotted in 30 minutes
- 2 Plasma + medium quartz clotted in 7 minutes
- 3 Plasma + dry quartz clotted in 8 minutes

In a second series of experiments centrifuged plasma obtained from negative phase blood and containing a reduced number of platelets was similarly treated, except that the temperature conditions were those of the room throughout. One example will suffice for illustration.

- 1 Plasma control coagulation in 1 hour
- 2 Plasma + medium quartz clotted in 10 minutes.
- 3 Plasma + medium quartz clotted in 12 minutes.

An interesting variation of the last experiment was to use plasma from blood which had been deprived of practically all its platelets. As Burke and Tait have shown, such blood can be obtained by giving rapidly a minimal lethal dose of highly active tissue extract. Two examples are given.

*Exp D*

- 1 Plasma control no coagulation in 1 hour
- 2 Plasma + medium quartz no coagulation in 1 hour

*Exp E*

- 1 Plasma control no coagulation in 2 hours.
- 2 Plasma + medium quartz filmy coagulation in 15 minutes, no further coagulation.

As proved by the firm coagulation that resulted from addition of tissue extract, both these samples of blood contained abundant fibrinogen.

The net conclusion from all these experiments is that admixture of quartz particles of sufficient size, or in sufficient amount if excessively fine, causes prompt coagulation of drawn blood, provided the thrombocytes are present. By contrast the negative effect of mingling the same particles with the circulating blood is rendered more striking.

(c) *Fall in number of platelets on injection of particles*

Various workers have shown that when foreign particles are injected into the blood vessels of a mammal they are rapidly swept out of the blood stream. Thus in the experiments of Tait and McCartney<sup>(10)</sup>, who injected Indian ink, the blood was found to be free of particles 1 hour after injection, and so were the organs with the constant exception of the spleen, liver and bone marrow, and with the occasional exception of the lung and of the kidney. It is naturally a hard matter to determine by simple inspection of the blood the presence or absence of quartz particles at any specified time after injection. There is, however, no doubt that they are rapidly removed, because they can be detected in sections of liver, spleen and bone marrow at least as early as 15 minutes after injection.

As our object was to look for some relation between the blood platelets and the injected particles, platelet counts were carried out at various intervals after injection. It was at once rendered plain that introduction of quartz into the vessels causes an abrupt fall in the number of circulating platelets. In this respect our findings were similar to those of Burke and Tait<sup>(3)</sup>, who injected cell *débris*. Control experiments showed that the effect is in no way due to injection of glucose, it is wholly the result of mingling foreign particles with the blood. Soon after the initial fall the number of platelets begins to rise and after a period is again restored to normal.

For counting the platelets the method of Spitz<sup>(11)</sup> was used, the blood being diluted ten times. As a unit quantity of blood for enumeration we took the amount of the dilution that covered 16 squares of a Thoma-Zeiss hæmatocytometer. Some figures are appended representing the relative number of platelets in the blood before, and at different intervals after, injection of quartz, the experiments being grouped into three sets, according as enumerations were made at intervals of minutes, of hours or of days respectively after injection.

## Set I. (Time in minutes.)

Weight of rabbit, 1865 grm	Coarse quartz	Dose, 0.5 grm per kg	in 25 c.c. fluid.
Initial count	250	After 3 mins	55
Weight of rabbit, 1742 grm	Fine quartz.	Dose, 0.3 grm. per kg	in 22 c.c. fluid.
Initial count	274	After 10 mins	28
After 3 mins	21	" 20 "	85

From the above two experiments it appears that whether a small quantity of fine or a larger quantity of coarse quartz is used, a prompt fall in the number of circulating platelets results.

## Set II. (Time in hours)

Weight of rabbit, 2460 gm.	Fine quartz.	Dose, 0.02 gm. per kg	in 20 c.c. fluid.
Initial count	125	After 3 hours	75
After 3 mins.	27	" 4 "	77
" 1/2 hour	76		

Weight of rabbit, 1742 gm.	Fine quartz.	Dose, 0.03 gm. per kg	in 22 c.c. fluid.
Initial count	273	After 24 hours	156
After 3 mins.	19	" 48 "	152
" 6 hours	120	" 72 "	157
" 12 "	137		

Weight of rabbit, 2500 gm.	Fine quartz.	Dose, 0.008 gm. per kg	in 7 c.c. fluid
Initial count	200	After 1/2 hour	182
After 3 mins.	156	" 1 1/2 hours	170
" 1/2 hour	160	" 3 1/2 "	200

The second experiment of the set shows that when a considerable quantity is injected recovery may still be incomplete after 3 days. In the third experiment, where, after a small dose, the initial fall was slight, recovery soon occurred.

## Set III (Time in days.)

Weight of rabbit 1580 gm.	Medium quartz.	Dose, 0.32 gm. per kg	in 39 c.c. fluid.
Initial count	200	After 4 days	98
After 1 day	96	" 8 "	256

Weight of rabbit, 2040 gm.	Medium quartz.	Dose, 0.17 gm. per kg	in 27 c.c. fluid.
Initial count	208	After 4 days	67
After 3 mins	27	" 7 "	200
" 1 day	45	" 9 "	256

In these instances recovery was in each case delayed for a number of days. As other experiments have shown, the ultimate over-stepping of the initial figure in these two experiments is not a constant finding.

As it was possible that the blood drawn from the marginal vein of the ear might not be representative of the systemic blood, we carried out an experiment in which the samples for examination were drawn simultaneously from the ear vein and from the carotid artery. The rabbit was anaesthetised with ether, its carotid artery was exposed and clamped with the fingers. The artery was incised and a small quantity of blood was removed for examination. Almost at the same time another sample was taken from the ear vein. The arterial sample showed 168 platelets per unit, the ear samples showed 144 per unit. Of medium suspension 0.14 gm. per kg. was now injected. Blood removed 3 minutes later from the carotid artery showed 46 platelets per unit, blood removed almost simultaneously from the ear vein showed 33. Fifteen and 30 minutes respectively after the first samples were taken, blood removed from the carotid artery showed 42 and 50 platelets per unit. By this experiment it was rendered plain that as far as number of platelets

is concerned blood taken from the ear is a sufficient index of the condition of the general systemic blood

By repetition of the quartz injections the platelets can be kept at a low figure, but if the procedure is continued it leads to a fatal result. Thus in one experiment, in which the initial number was 138, a preliminary injection of 0.02 gm per kg of fine quartz caused an immediate drop to 30. Thereafter the platelet figure rose, being 79 at the end of  $1\frac{1}{2}$  hours. At the end of 4 hours, when the figure was 86, another dose of similar amount was injected. This time there was no profound drop, the platelet count 3 minutes later registering 82. After a lapse of 21 hours from the time of the first injection, the platelet number was found to be 48. A dose of 0.005 gm per kg was now given, whereupon the figure fell to 22. A fourth injection, again of 0.005 gm per kg, was administered 2 hours later. The animal soon showed marked weakness and died 3 hours later, its blood platelet number being very low.

The results cited above refer in each case to injections of quartz particles. Similar phenomena occur when other particles, *e.g.* barium, carmine or Indian ink, are substituted for quartz. As the particles can be shown to come to rest in the spleen, liver and bone marrow, and as a large number of platelets disappear simultaneously with the particles, the presumption is that the platelets, having ingested or having adhered to the particles, also come to rest in the same organs. It is a difficult matter to provide conclusive proof that the platelets which disappear have found a resting place in these localities. As some subsidiary evidence pointing in this direction we may mention that E. S. Mills, working in this laboratory on phagocytosis of Indian ink by the ellipsoids of the spleen, finds in these organs individual aggregations of ink of the size of platelets. The recovery in number of circulating platelets after an injection of foreign particles is, we believe, due to the appearance of a fresh crop.

#### (d) *Relation to coagulation*

It remains to discuss the anomaly of the first two experimental sections, according to which extravascular admixture determines coagulation, whereas intravascular admixture, even with coarse particles, does not nearly so readily do so. The blood in circulation must in some way be protected against a coagulating influence which, if allowed access to drawn blood, is sufficient to induce irreversible solidification. The probability is that a partial transformation of fibrinogen into fibrin occurs in the circulating blood, but under conditions that preclude general solidification.

A closely similar anomaly arises in connection with the action of tissue extract or "thromboknase" Direct addition of this material to drawn blood invariably causes coagulation, yet if the substance is very slowly injected into a vein the circulating blood stays fluid and soon exhibits impaired coagulability or "negative phase" Though it has failed to undergo solidification, such negative phase blood, as J Mellanby<sup>(20)</sup> showed, has been wholly or partially defibrinated Mellanby's explanation of the phenomenon is that when the kinase is slowly injected fibrin actually forms, but so gradually and in such small molecules that time and opportunity are allowed for its removal by means of the tissue cells If the injection is more rapid, large cohering filaments form, which in turn implies a real thrombosis

It is probable that the quartz injection, like a slow injection of thromboknase, leads to transformation of fibrinogen into fibrin. Quartz, however, acts in a different way from thromboknase Whereas the latter directly coagulates fibrinogen (see Tait and Green<sup>(16)</sup>) the action of the quartz is indirect It first causes cytolysis of platelets, owing to this cytolysis thrombin is liberated, the thrombin then acts upon the fibrinogen It is open to suppose that the process of thrombin liberation may occur to a considerable extent in the blood stream At the same time we have reason to believe that a large number of platelets, which have adhered and are about to cytolysse, are caught by the cells and other structures of the reticulo-endothelial system, whereby they may be put out of action, so far at least as power to cause generalised coagulation is concerned In any case, owing to its indirect mode of action, an injection of quartz particles must operate on fibrinogen more slowly than a corresponding injection of thromboknase

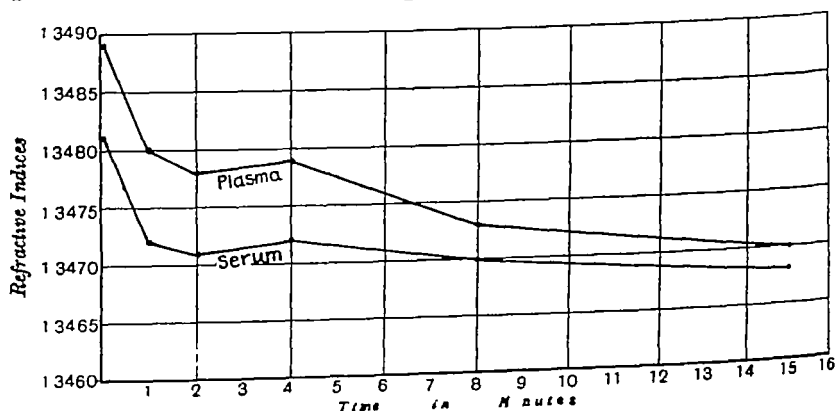
At some particular point, say, 10-20 minutes, after a single injection of quartz, and while abundant particles are still floating free in the blood, the coagulation time of the blood is found to be greatly shortened This can be determined by pulling a needle through the drawn blood and noting the moment of appearance of the first thread of fibrin, which, at this stage, may be almost immediate! This striking fact supports the idea that thrombin is then being liberated freely within the circulating blood. So rapid is the formation of fibrin at this stage that we had difficulty in understanding how the blood keeps fluid in the vessels We judged that the animal had reached a danger-point, at which the vascular mechanism for counteracting thrombosis was being called into strenuous activity This in turn throws additional light on the difference in action between an injection of tissue extract and one of



quartz In the case of tissue extract the outstanding experimental difficulty is to inject so slowly as to avoid, at any stage, sudden overloading of the mechanism for counteracting thrombosis With quartz it is the other way about, one may approach, but one does not readily reach the overloading point

At a later stage, say, after 40 minutes, the coagulation time becomes much greater than normal, and the drawn blood is slow to clot on glass and other water-wettable receptacles It does, however, coagulate solidly in the end, which proves that it still contains fibrinogen The delay in spontaneous coagulation at this stage is no doubt associated with the reduction in number of platelets, and perhaps with a difference in physiological state of the new crop as compared with the old

In order to discover whether fibrinogen is removed from the blood as a result of quartz injection, we had recourse to the refractometer At different stages after injection samples of blood were withdrawn These were divided into two portions One was first oxalated and then centrifuged in order to provide plasma The other, which was left to clot, provided the serum The accompanying figure represents in graphical



Graphs of refractometer readings of plasma and of serum after an injection of quartz particles

form the result of one of these experiments The abscissa represents time in minutes after injection, the ordinates represent the refractometer readings The upper trace shows readings of the plasma, the lower trace readings of the serum It will be seen that during the first minute there is in each case a sharp and equal fall of the respective refractive indices, due to blood dilution Thereafter the course of each graph is more nearly linear During this second stage the refractive index of the serum falls

off slightly, probably owing to successive withdrawals of blood samples. That of the plasma, on the other hand, falls more rapidly, at the end of 15 minutes it registers only 0.0002 above the serum, whereas at the moment of injection it stood 0.0008 above the serum. This plainly indicates a loss of fibrinogen from the plasma.

Just as Mellanby had to account for the disappearance of fibrinogen in negative phase blood on the assumption that fibrin had actually been formed and removed from the blood, so we have here to postulate the activity of a definite anti-thrombotic mechanism, as opposed to a material "anti-thrombin." As Mellanby pointed out, conditions tending towards thrombosis, *e.g.* death or injury of blood cells, are continually arising in the blood. Should the organism be unable to deal with a slow (or, we may add, dispersed) formation of fibrin in its circulating blood, thrombosis would be a continual occurrence in the life-history of animals. We shall not here attempt to define the mechanism in question or to specify its conditions of operation. Suffice it to say that the reason why a direct injection of thrombin into the circulation fails to cause thrombosis, whereas injection of thrombokinase is effective, probably depends upon the difficulty of securing an adequate concentration of thrombin to overwhelm for the moment the anti-thrombotic mechanism.

It may be mentioned that during the course of this investigation one of the authors found that injection of particles affects not only the number of platelets, but also the number and kind of red cells in circulation (see Elvidge(4)).

### SUMMARY

1 *A priori* considerations seemed to indicate that an injection of very fine inert particles might fail to cause coagulation of the circulating blood, whereas an injection of coarse particles might cause coagulation. It was found that an intravascular injection even of coarse particles does not necessarily determine thrombosis.

2 Addition of coarse particles to paraffin-held, drawn blood, by causing cytolysis of thrombocytes, eventually causes coagulation. Addition of an adequate quantity of fine particles to the drawn blood of the frog similarly causes coagulation, while addition of a smaller quantity of fine particles does not.

3 As the result of an intravascular injection of particles a fall in the number of circulating platelets occurs. After a heavy injection the fall is profound (though complete disappearance was never observed), and some days must elapse before the previous number is restored. With

injection of a smaller amount the fall in number of platelets is less, and the time for recovery of the initial number is shorter. By repetitions of the injections at intervals less than the time necessary for recovery, the number of circulating platelets can be kept low.

4. Admixture of particles with the circulating blood, while failing to cause thrombosis, nevertheless effects a transformation of fibrinogen into fibrin. The reason why thrombosis does not occur is that the particular conditions of formation of the fibrin allow of a successful reply on the part of the natural "anti-thrombotic mechanism" of the circulation.

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# STUDIES ON THE INTERNAL SECRETIONS OF THE OVARY II The effects of injection of the œstrus producing hormone during pregnancy

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## I INTRODUCTION

It has been shown elsewhere (Parkes(6)) that the ordinary cyclic occurrence of œstrus in the adult female mouse is maintained in the absence of follicles and corpora lutea and hence that the length of the diœstrous period in the unmated female mouse is not regulated by the length of the functional life of the corpus luteum of ovulation. At the same time, since œstrus does not occur during pregnancy or during lactation, it is necessary to suppose that an œstrus inhibitor action is set up when the corpus luteum is caused to persist by either of these events (see, for instance, Loeb(4)). The exact nature of this inhibitor action is not clear, but it is probably hormonal. As we have pointed out in a previous paper(7), it is fairly clear that œstrin<sup>1</sup> is being constantly produced by the ovary, so that the œstrus inhibitor action of the persistent corpus luteum is presumably obtained by delaying the attainment of the threshold required for the production of œstrus.

In any given animal, therefore, the persistent corpus luteum must be capable of dealing with the amount of œstrin produced by the ovary during the time when the corpus luteum is dominant, but nothing appears to be known of the margin between the œstrus-producing stimulus and the œstrus-inhibiting action of the persistent corpus luteum.

Experiments were, therefore, designed to throw light on this problem by injections of œstrin during the functional life of the persistent corpus luteum, with the object, so to speak, of confronting the corpus luteum with a much greater œstrus-producing stimulus than it is normally called upon to deal with. Since functional persistent corpora lutea are necessary to suppress œstrus during pregnancy, and also apparently to

<sup>1</sup> In a previous paper we have pointed out that the œstrus producing hormone is not confined to the follicle, and that, therefore, 'the term œstrin' would be preferable to 'folliculin'.

maintain gestation, overriding of the corpus luteum by an artificial excess of œstrin might be expected to result in the abnormal appearance of œstrus and in the termination of the pregnancy

The work recorded in this paper deals with the effects of œstrin injections during pregnancy. The effect on œstrus inhibition during lactation will be dealt with in a later communication

*Preparation of œstrin* The preparation of œstrus-producing extracts from ovaries was dealt with in the previous paper, and substantially the same methods have been used in preparing extracts for injection during pregnancy. In most cases the crude product, as before, was emulsified with 1 p c  $\text{Na}_2\text{CO}_3$  and tested immediately, but where it was required to have emulsions potent in small amounts a portion of the yield was emulsified and tested and the M U G (mouse units per gram of crude yield) calculated. The remainder of the yield was then emulsified with an amount of carbonate sufficient to produce an emulsion containing 1 M U in some known small amount. It has been found that alkaline emulsions kept for long periods tend to become less active, and the emulsions were, therefore, used as fresh as possible

*Detection of œstrus* The detection of œstrus in mice is comparatively simple since Allen(1) investigated the cyclic changes in the nature of the vaginal smear. This technique, as applied to the mouse colony in question, has been dealt with elsewhere(5) and need not be described in detail here. It may, however, be said that the fact that the periods of vaginal cornification brought on artificially during pregnancy were normally accompanied by copulation gives further evidence of the reliability of this technique

*Detection of pregnancy* In the mouse the accurate detection of pregnancy before the 9-10th day is at present impracticable. The "placental sign" in the vagina appears at about this time(5) and careful palpation of the abdomen will usually reveal the presence of the nodules in the uterus. From the 12th day onwards the embryos may be readily palpated. During the first week, however, the occurrence of pregnancy can be assumed on a probability basis. The vaginal plug, indicating copulation, can be readily detected, and it has been found that of fertile females mated with fertile males some 75 p c produce a litter following the discovery of the plug. Since the number of litters conceived is undoubtedly greater than the number born, this figure may be taken as a conservative estimate of the percentage conceptions. The following experiments, therefore, relating to injections in the first 9 days when pregnancy cannot be detected in the intact animal have been based on

the probability that three out of four animals become pregnant after copulation. During the later stages when pregnancy can be detected, injections were only made into animals known to have become pregnant after copulation.

*Scheme of experiments* Injections of oestrin carried on over a number of days were begun at various stages of gestation. They may, however, be grouped into three main periods, (a) at 2 days after copulation, when the corpus luteum is comparatively young and the embryos not properly imbedded, (b) at 3-8 days after copulation, when pregnancy can still not be detected with certainty, and (c) after the 10th day, when the corpus luteum has reached its maximum development and when any results may be observed on the genitalia of the young (in the absence of abortion). The effects of injection have been noted (a) by the history of the gestation, (b) by the nature of the vaginal smear and the re-occurrence of oestrus. In the normal way no cornification of the vagina is to be found during pregnancy and no copulation occurs.

## II INJECTION AT THE SECOND DAY

Since not every animal becomes pregnant after copulation, and since it is known that the course of gestation in the very early stages is easily disturbed, large-scale control experiments were first of all performed. The percentage probability of pregnancy occurring after copulation was already known and, as mentioned above, was about 75 p c. The effects of daily injection were studied by means of the following blanks: (a) injection of inactive ovarian extracts<sup>1</sup>, (b) injection of beef extract prepared

TABLE I. Result of injections of inactive ovarian extracts beginning 2 days after copulation.

No of mouse	Days injected	Amount per day c c.	Results	
FH 19	16	5	No litter	Copulation at 18th day
FH 20	7	5	"	" 9th "
FH 21	9	5	"	" 11th "
FH 22	7	5	"	" 8th "
FH 23	8	5	"	" 9th "
FH 24	8	5	"	" 9th "
FH 25	5	5	"	" 7th "
FH 26	6	5	"	" 8th "
FH 27	7	5	"	" 8th "
FH 31	10	5	"	" 12th "
FH 32	9	5	"	" 10th "
FH 33	5	5	"	" 6th "
FH 34	4	5	"	" 5th "

<sup>1</sup> These were extracts which, though originally oestrus producing active, had become practically inactive (probably owing to prolonged emulsification in alkaline media)

by the same method, (c) injection of cholesterol emulsions, and (d) injection of distilled water. The results were as above (Table I)

With regard to these results the following remarks may be made

(a) Since in none of the 13 cases did pregnancy occur from the first copulation, it is clear that the injections had the effect of terminating the gestation. (At least nine of these mice should have become pregnant.)

(b) In all cases a further period of œstrus and copulation was observed at varying times after the beginning of the injections. It is clear, however, that this further period of œstrus had only an indirect connection with the injections, *i.e.* pregnancy having been abolished, the œstrous cycle started again in the normal way after the involution of the corpus luteum. The average time elapsing before the reappearance of œstrus (9.2 days) is very near the normal length of the diœstrous period after sterile copulation (Parkes (5)).

(c) The normality of the œstrous period occurring after injections is also shown by the fact that the majority of the animals became pregnant as the result of the second copulation.

(d) It seems clear, therefore, that the course of events in these animals was that the injections caused the pregnancy to terminate, that this resulted in the corpus luteum failing to become persistent and in the subsequent reappearance of œstrus, followed by copulation and pregnancy.

The exact nature of the means whereby the injections disturbed the pregnancy was not apparent, and further experiments were carried out. The results are summarised below.

TABLE II. Effects of various injections 2 days after copulation.

Material injected	No. of animals	Amount injected daily for 5-7 days c c	Results	
			No litters	Litters
Extract of lean beef	14	5	9	5
Cholesterol emulsion	4	5	3	1
Distilled water	7	5	5	2
	5	1	2	3

From the results on these 30 animals it may be concluded that the injection for 5-7 days from the 2nd day onwards of 5 c c of fluid materially lowers the probability of pregnancy becoming established, whereas injection of 1 c c daily does not. In view of this conclusion it was clear that the active ovarian extracts used for injection during pregnancy should be sufficiently pure to allow of the necessary mouse units being injected in small amounts of fluid.

Following this preliminary work the following injections of œstrus-producing active ovarian extracts were made

TABLE III. Injection of active ovarian extracts beginning 2 days after copulation.

No of female	Day injected	Volume per day (c.c.)	Total M.U. given	Results
FH 79	2	1	1.0	Copulation at 4th day Cornification at 5th day
FH 80	4	1	2.0	Copulation at 6th day Cornification at 5th day
FH 81	5	1	2.5	Cornification at 5th day
FH 82	6	1	3.0	Cornification at 6th day
FH 83	2	1	1.0	Copulation at 4th day
FH 84	3	1	1.5	Copulation at 5th day
FH 85	6	1	3.0	Cornification at 5th day
FH 92	4	3	4.0	Copulation at 6th day
FH 93	2	3	2.0	Copulation at 4th day
FH 94	3	3	3.0	Copulation at 5th day
FH 95	2	3	1.0	Cornification at 3rd day

In these cases the daily injections were continued till copulation took place or until cornification of the vaginal epithelium was observed, and since one or both of these happened in all cases within 6 days of the previous copulation it must be concluded that the doses given, in some cases as low as one mouse unit were sufficient to override the corpus luteum and produce a new œstrous period. That this result is actually due to the presence of the œstrus-producing substance in the extract is shown by two fundamental points of difference from the results brought about by the mere injection of fluid

(a) In the first seven cases the amount of fluid injected was not of an order which had been found sufficient to disturb the pregnancy and allow of a further œstrous period to occur

(b) The œstrous periods following the injection of active extracts occur much more quickly (3-6 days after previous copulation) than do those occurring in the normal course of events after sterile copulation or after the disturbance of pregnancy by the injections of large amounts of fluids

It may be concluded, therefore that the corpora lutea of early pregnancy can be overridden by the injection of amounts of œstrin represented by 1-3 mouse units, and it must, therefore, be assumed that the œstrus inhibitor action of the young corpora lutea is capable of dealing with only a limited œstrus-producing stimulus in addition to that already provided by the ovary. The induction of œstrus seems to lead automatically to the destruction of the embryos

In order to ascertain whether any histological changes were brought about by this artificial induction of œstrus, ovaries were obtained from



animals at the time of the occurrence of the induced œstrous period. Two possible histological effects might have been expected, (a) retrogressive changes in the corpora lutea, and (b) unusual maturation of follicles. Histological examination of these ovaries, however, showed that little difference, if any, from ovaries of the same time of pregnancy was to be observed. Certainly it appeared from the sections that ovulation had not accompanied the induced œstrous period, and this supported the deduction made from the fact that in no case did pregnancy result from copulation at this time.

This question of the stimulation of the follicles by œstrin injections is of some importance from the point of view of clinical application. Since all the evidence goes to show that menstruation is the result of pseudo-pregnant degeneration it does not seem probable that it will be possible to bring about menstruation in cases of amenorrhœa as a direct result of œstrin injections. It might, however, be supposed that injection would lead to stimulation of follicles and thence to ovulation and to the production of the corpora lutea which appear to be responsible for the pre-menstrual congestion of the uterus. Further work is now being done with regard to the possibility of stimulating follicular development by means of œstrin injections.

### III INJECTION AT 4-10TH DAY

Control injections of extract of lean beef beginning 4-10 days after copulation showed that at this stage the injection of fluids does not materially affect the probability of copulation resulting in detectable pregnancy. The details of the controls are as follows.

TABLE IV Control injections beginning 4-10 days after copulation

No of female	Age of pregnancy	Days injected	Amount per day (c.c.)	Results
FH 66	6	6	5	Autolysis discovered at 14 days
FH 67	6	6	5	
FH 68	6	6	5	"
FH 69	4	6	5	"
FH 70	4	6	5	"
FH 71	7	5	5	"
FH 72	7	5	5	No pregnancy
FH 73	7	5	5	Normal pregnancy
FH 74	7	5	5	No pregnancy
FH 5	10	4	5	Litter of 7
FH 6	10	4	5	Litter of 5

Thus of the eleven animals injected eight proceeded to normal pregnancy, and this degree of failure to produce a litter after copulation is not materially greater than that found in untreated animals.

Injectations of active ovarian extracts, however, led to the failure of seven out of nine animals to produce a litter. The details are as follows

TABLE V Injection of active ovarian extracts beginning 4-8 days after copulation.

No of female	Age of pregnancy	Days injected	Total M.U. given	Results
FH 10	6	4	2.0	Litter of 9
FH 11	6	4	2.0	Reabsorption
FH 12	7	5	2.5	Litter of 7
FH 13	7	5	2.5	No litter
FH 89	7	5	2.5	Copulation at 13th day
FH 4	9	4	2.0	Aborted remains at 17th day
FH 91	8	5	2.5	Copulation at 14th day
FH 1	10	5	2.5	No litter
FH 28	10	5	2.0	No litter Copulation at 14th day

Since seven out of nine of these animals failed to have litters, it would appear that an œstrus-producing stimulus equal to 2-2.5 mouse units can, in some cases, though not invariably, override the corpora lutea at this stage, cause œstrus to appear and terminate the pregnancy.

#### IV INJECTION AT 11-18TH DAY

During this stage pregnancy is definitely detectable, both by the vaginal smear and by palpation of the fetuses, and, since only animals found to be pregnant were used, no allowance is necessary for the normal percentage of infertile copulations. In addition, a number of control experiments showed that large amounts of beef extract and cholesterol emulsion could be injected without affecting the course of the pregnancy. The effects of injection of œstrin could therefore be determined with some certainty in the second half of pregnancy, and the following table (arranged in ascending order of mouse units injected) shows the results of injections of various amounts at a variety of times.

Considering the delicate nature of such experiments the results recorded above are remarkably consistent. Of the 16 pregnant animals receiving 2.5 and less mouse units only one failed to produce a litter at full time, and only one of the litters born showed abnormality (still-born). Animals receiving 3.0 mouse units and over, on the other hand, were mostly affected, six out of the eight pregnancies terminating abnormally in abortion or re-absorption.

None of the animals which ultimately produced litters showed any vaginal signs of œstrus following the injections, and in no case did copulation take place unless accompanied by abortion or re-absorption. In cases where the pregnancy terminated following large injections of œstrin signs of œstrus were not in all cases detected, but even if such

TABLE VI Injections of oestrin during second half of pregnancy

No of female	Age of pregnancy	Days injected	Total m u given	Results
FH 63	18	1	5	Litter of 3 at normal time
FH 65	15	3	1.5	" 6 "
FH 3	11	3	1.5	No litter
FH 14	15	4	2.0	Litter of 6 at normal time
FH 15	15	4	2.0	" 7 "
FH 16	15	4	2.0	" 7 "
FH 17	14	4	2.0	" 7 "
FH 18	14	4	2.0	" 8 "
FH 99	15	3	2.0	" 5 "
FH 2	11	4	2.0	" 6 at 20 days
FH 29	12	8	2.0	" 2 at normal time
FH 30	12	8	2.0	" 8 "
FH 7	11	5	2.5	" 6 "
FH 8	11	5	2.5	" 6 "
FH 9	11	5	2.5	" 7, stillborn
FH 51	14	5	2.5	" 4
FH 52	13	6	3.0	Autolysing foetuses aborted
FH 64	12	6	3.0	Foetuses re absorbed
FH 87	15	3	3.0	Litter of 3 at normal time
FH 88	15	3	3.0	Re absorption
FH 77	14	4	4.0	Abortion and copulation
FH 86	14	4	4.0	Re absorption
FH 78	14	5	5.0	Litter of 11
FH 90	13	5	5.0	Abortion of autolysing foetuses

signs had occurred in all cases, the vaginal debris resulting from autolysis of the foetuses would frequently have made detection impracticable

One further point remains to be considered. Courrier<sup>(3)</sup> has reported that injections of "folliculin" (oestrin) into pregnant guinea pigs lead to changes in the foetal uteri similar to those characteristic of oestrus in the adult. With a view to investigating this point on the mouse full-time foetal uteri were examined after injection of the mother. The animals examined were from the litters of FH 65, FH 78 and FH 87, females which had received respectively 1.5, 5 and 3 mouse units. The uteri showed not the least difference from normals, or from each other, in spite of the variation in the dosage of the mothers.

The uterus of the normal female at birth is about 25 mm in diameter, and the wall of the uterus, including uterine epithelium and peritoneal covering is some 80  $\mu$  thick. The uterine epithelium is for the most part only one cell thick, though in odd places it may be two cells. The stroma is 10-15 cells thick. The uteri of young from treated mothers showed no thickening of the epithelium or oedema, and were in every way identical with normals. It would appear, therefore, that injection of the pregnant mouse with oestrin does not produce in the foetal uteri any changes which might be considered analogous to the oestrous changes in the adult uterus.

## V DISCUSSION

The following are the chief results which have been arrived at and which need to be discussed

(1) Injection of oestrin in sufficient amounts will always cause the rapid appearance of oestrous symptoms in the early stages of pregnancy and very often in the later stages

(2) The amount required is, however, much greater (roughly twice as much) in the later stages of pregnancy than in the early ones

(3) The artificial production of oestrous symptoms during pregnancy is, however, incompatible with the continuation of the pregnancy

(4) Where injections insufficient to terminate the pregnancy are given young are born normally, the males showing no abnormality of sex differentiation and the females no oestrous characteristics

From previous work<sup>(7)</sup> it has been concluded that the cyclic action of oestrin is brought about by the periodic attainment of a threshold value, and that the persistent corpus luteum has the effect of delaying the attainment of this threshold and of throwing the cycle out of gear, as during pregnancy, pseudo-pregnancy and lactation. In any case it may be said with some confidence that the persistent corpus luteum is responsible for the non-occurrence of oestrus during pregnancy. At the same time oestrin can be readily extracted from ovaries of pregnant animals, so that it is necessary to suppose that the oestrus inhibitor action of the corpus luteum exerts a dominating action during pregnancy<sup>1</sup>. The exact nature of this inhibitor action is not yet clear, but it is plain that it is not localised to the ovary itself. In animals where only one ovary ovulates at a time, for instance, the presence of a corpus luteum in one ovary is sufficient to stop the oestrus-producing stimulus of the other as well.

The results described above suggest that the normal balance during pregnancy of corpus luteum dominance has been reversed by the injections of oestrin, and that a less external supply is required to do this during the early stages than during the later stages. Why this should be so is not absolutely clear, but the greater maturity of the corpora lutea during the last half may increase their oestrus inhibiting potentialities. The problem of why the artificial induction of oestrus should

<sup>1</sup> The fact that during pregnancy or lactation it is the persistent corpus luteum which exerts the influence and not the mere fact of pregnancy or lactation *per se* is shown by the fact (Long and Evans) that removal of the young during lactation results first in changes in the corpora lutea and then in the appearance of oestrus.

invariably terminate the pregnancy is also obscure, but two suggestions may be made

(a) It is possible that local disturbance of the uterus consequent upon the effort to assume an œstrous condition would render the position of the embryos untenable

(b) Since the œstrus inhibiting function of the corpora lutea is overridden it is possible that the function relating to the maintenance of pregnancy is also suppressed<sup>1</sup>

On the whole, the latter view seems the most probable, especially in view of the fact that re-absorption rather than abortion is the usual result of œstrin injections

These results have a possible application to medicine and animal breeding. In cows the undue persistence of corpora lutea is responsible for a type of sterility which is now cured in many cases by squeezing out the corpora lutea *per rectum*. It would seem, however, that the injection of œstrin would override the inhibitor action of the persistent corpus luteum and as a result of this would indirectly lead to ovulation and to the restoration of fertility

In the same way, a type of amenorrhœa seems to occur in the human subject which is due to the failure of the corpus luteum to retrogress, and which is analogous to prolonged pseudo-pregnancy. In such cases the overriding of the corpus luteum (assuming the œstrus inhibiting factor to be the same as that causing pre-menstrual congestion of the uterus) by œstrin injections should lead to the reappearance of the menstrual cycle

The last point to be considered is the immunity of the foetal uteri to œstrin injected into the mother. At first it is natural to suppose that the sex hormones of the mother do not pass across the placenta, other wise it is difficult to see how the differentiation of the male foetuses can proceed undisturbed. We have, however, secured fairly definite evidence that œstrin at least can cross the placenta. A further possibility is that, since foetal uteri can only be subjected to a maternal injection of œstrin less than that required to override the corpora lutea, the œstrus inhibiting action of the corpus luteum also extends to the foetus. This idea, however, implies that the foetal organism is normally under the influence of corpus luteum secretion during gestation. Probably the most reasonable theory, in view of the very definite absence of effect on the foetal uteri of œstrin injections of the mother, is to suppose that the foetal

<sup>1</sup> Removal of the corpora lutea during pregnancy, especially in the early stages, normally results in the termination of the pregnancy

organism is incapable of responding to oestrous stimulation. This hypothesis has been confirmed by the finding that the injection of 1 mouse unit of oestrin into new-born female young does not appear to produce any changes whatever in the uteri.

## VI SUMMARY

(1) The injection of oestrin during the early stages of pregnancy invariably causes the rapid reappearance of oestrus and the termination of the pregnancy.

(2) During the later stages of pregnancy injection in sufficient amounts will nearly always terminate the pregnancy, but the amount required is much greater (about twice as much).

(3) In cases where litters are born after injection of the mother during the later stages of pregnancy no abnormality in sex differentiation occurs in the males, and no oestrous symptoms are observed in the females.

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# MYOTHERMIC OBSERVATIONS ON THE DOGFISH

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IN subsequent papers Lehnartz will describe experiments on several muscles of different animals, frog, toad, tortoise and mussel, relating the absolute values of heat and tension for various durations of stimulation. It was obviously desirable to extend the range of such observations, and the opportunity was taken of a visit to the Marine Biological Laboratory at Plymouth to work with the muscles of the dogfish, which survive well after the death of the animal. Two muscles of the lower jaw were employed, both admirably suited to myothermic experiments, a single muscle, *coracomandibularis*, and a double one, *coracohyoideus*, of which usually only one of the pair was used. The thermopile was of the type described in (1) Fig 1, and observations were made throughout in air. Small dogfishes only were used. In some experiments the muscle was left on the thermopile in air during the preliminary temperature equalisation; in others it was left in a solution of the composition<sup>1</sup> NaCl 34 M, CaCl<sub>2</sub> 0.09 M, KCl 0.06 M, urea 34 M, until shortly before the readings were commenced. The latter treatment seemed to leave the muscles in rather better condition, although never so active as they were immediately after removal from the animal. They are very easily excited, a maximal contraction being obtained with the secondary of a Harvard coil removed as far as possible from the primary and tilted to 45° (2 volts in primary, tetani of various durations adjusted by a Lucas revolving contact-breaker). The initial heat-production only was determined, from the deflection of a sensitive moving-magnet galvanometer, the tension developed being simultaneously recorded on a slowly moving drum.

These muscles react very quickly, and when the stimulus was prolonged beyond 1 sec (often 0.5 sec) the mechanical response showed signs of "tailing off." Experiments were made only over the range within which no great "tailing off" occurred.

<sup>1</sup> Recommended by Prof. A. J. Clark as suitable for dogfish hearts.

Calibration was carried out in the dead muscle by a strong faradic current from the coil liberating a known amount of heat in it. The heating effect ( $C^2t$ ) of this current was determined by passing it at the same time through a calibrated arrangement consisting of a resistance wire wound round the junctions of a thermopile connected to a moving-coil galvanometer. The resistance  $R$  of the muscle was determined by passing the same current through a resistance box in series with the hot wire, and adjusting the box until the same deflection (as when the muscle was in the circuit) was obtained on the moving coil galvanometer. The heat liberated in the muscle is then  $RC^2t$  joules.

The same effects (on heat and tension) of varying the length are found as in frog or tortoise (1). Preliminary trials were made in all experiments in order to ascertain, and work at, the length at which  $H/Tl$  was a minimum.

Observations were made in a series of increasing durations of stimulus and were repeated in reverse order, a mean being taken at each duration.

In the first experiment given below no great "tailing off" of the tensions occurred with 1 sec of stimulation, and the muscle remained in good condition, so that a second (double) series of readings could be made, agreeing well with the first.

*Exp 1. Coracomandibularis of dogfish in air Room temperature*

1. Time of stimulation (seconds)	02	05	10	30	60	1 00	2 00
Rise of temperature (1/1000° C)	1 7	2 1	3 2	6 0	9 6	13 1	19 3
Tension developed (gm. weight)	24	30	37	45	48	48	48
$H/Tl$	32	37	40	62	93	1 27	1 86
2. Time of stimulation (seconds)	02	05	10	30	60	1 00	2 00
Rise of temperature (1/1000° C)	1 5	1 9	2 8	5 3	8 1	11 1	15 1
Tension developed (gm. weight)	23	29	35	43	44	45	45
$H/Tl$	29	30	38	57	86	1 14	1 56
Mean $H/Tl$ both series	31	34	39	60	90	1 21	1 71

*Exp 2 Coracomandibularis of dogfish in air Room temperature.*

Time of stimulation (seconds)	02	05	10	20	50	1 0
$H/Tl$	38	40	51	61	93	1 30

Fig 1 shows the results. It will be seen that for short stimuli the value of  $H/Tl$  is between 0.3 and 0.4, slightly larger than the average for the frog, appreciably larger than for the tortoise, but of the same order of quantities as has been found in all muscles hitherto studied. It is obvious, of course, that  $H/Tl$  is not an absolute constant for muscle oncoming failure, or fatigue, tends to raise it, the action of fluoride (as Lehnartz will show) to lower it. It is probable that these dogfish muscles immediately after removal from the animal—when they certainly



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IN subsequent papers Lehnartz will describe experiments on several muscles of different animals, frog, toad, tortoise and mussel, relating the absolute values of heat and tension for various durations of stimulation. It was obviously desirable to extend the range of such observations, and the opportunity was taken of a visit to the Marine Biological Laboratory at Plymouth to work with the muscles of the dogfish, which survive well after the death of the animal. Two muscles of the lower jaw were employed, both admirably suited to myothermic experiments, a single muscle, *coracomandibularis*, and a double one, *coracohyoideus*, of which usually only one of the pair was used. The thermopile was of the type described in (1) Fig 1, and observations were made throughout in air. Small dogfishes only were used. In some experiments the muscle was left on the thermopile in air during the preliminary temperature equalisation; in others it was left in a solution of the composition<sup>1</sup> NaCl 34 M, CaCl<sub>2</sub> 0.09 M, KCl 0.06 M, urea 34 M, until shortly before the readings were commenced. The latter treatment seemed to leave the muscles in rather better condition, although never so active as they were immediately after removal from the animal. They are very easily excited, a maximal contraction being obtained with the secondary of a Harvard coil removed as far as possible from the primary and tilted to 45° (2 volts in primary, tetani of various durations adjusted by a Lucas revolving contact-breaker). The initial heat-production only was determined, from the deflection of a sensitive moving-magnet galvanometer, the tension developed being simultaneously recorded on a slowly moving drum.

These muscles react very quickly, and when the stimulus was prolonged beyond 1 sec (often 0.5 sec) the mechanical response showed signs of "tailing off". Experiments were made only over the range within which no great "tailing off" occurred.

<sup>1</sup> Recommended by Prof. A. J. Clark as suitable for dogfish hearts.

Calibration was carried out in the dead muscle by a strong faradic current from the coil liberating a known amount of heat in it. The heating effect ( $C^2t$ ) of this current was determined by passing it at the same time through a calibrated arrangement consisting of a resistance wire wound round the junctions of a thermopile connected to a moving-coil galvanometer. The resistance  $R$  of the muscle was determined by passing the same current through a resistance box in series with the hot wire, and adjusting the box until the same deflection (as when the muscle was in the circuit) was obtained on the moving coil galvanometer. The heat liberated in the muscle is then  $RC^2t$  joules.

The same effects (on heat and tension) of varying the length are found as in frog or tortoise<sup>(1)</sup>. Preliminary trials were made in all experiments in order to ascertain and work at, the length at which  $H/TL$  was a minimum.

Observations were made in a series of increasing durations of stimulus and were repeated in reverse order, a mean being taken at each duration.

In the first experiment given below no great "tailing off" of the tensions occurred with 1 sec. of stimulation, and the muscle remained in good condition, so that a second (double) series of readings could be made, agreeing well with the first.

*Exp 1. Coracomandibularis of dogfish in air Room temperature.*

1. Time of stimulation (seconds)	02	05	10	30	60	1 00	2 00
Rise of temperature (1/1000° C)	1.7	2.1	3.2	6.0	9.6	13.1	19.3
Tension developed (gm. weight)	24	30	37	45	48	48	48
$H/TL$	32	37	40	62	93	1 27	1 86
2. Time of stimulation (seconds)	02	05	10	30	60	1 00	2 00
Rise of temperature (1/1000° C)	1.5	1.9	2.8	5.3	8.1	11.1	15.1
Tension developed (gm. weight)	23	29	35	43	44	45	45
$H/TL$	29	30	38	57	88	1 14	1 56
Mean $H/TL$ both series	31	34	39	60	90	1 21	1 71

*Exp 2. Coracomandibularis of dogfish in air Room temperature.*

Time of stimulation (seconds)	02	05	10	20	50	1 0
$H/TL$	38	40	51	61	93	1 30

Fig 1 shows the results. It will be seen that for short stimuli the value of  $H/TL$  is between 0.3 and 0.4, slightly larger than the average for the frog, appreciably larger than for the tortoise, but of the same order of quantities as has been found in all muscles hitherto studied. It is obvious, of course, that  $H/TL$  is not an absolute constant for muscle oncoming failure, or fatigue, tends to raise it, the action of fluoride (as Lehnartz will show) to lower it. It is probable that these dogfish muscles immediately after removal from the animal—when they certainly

develop greater tensions—would, if their heat-production could be measured, show appreciably lower initial values of  $H/Tl$ . In forming an impression, however, of the relative constancy of  $H/Tl$  for a short stimulus over a wide range of muscles and conditions we must bear in mind that it might, *a priori*, have had any value from zero to infinity, whereas actually it always lies, in muscles in reasonably good condition,

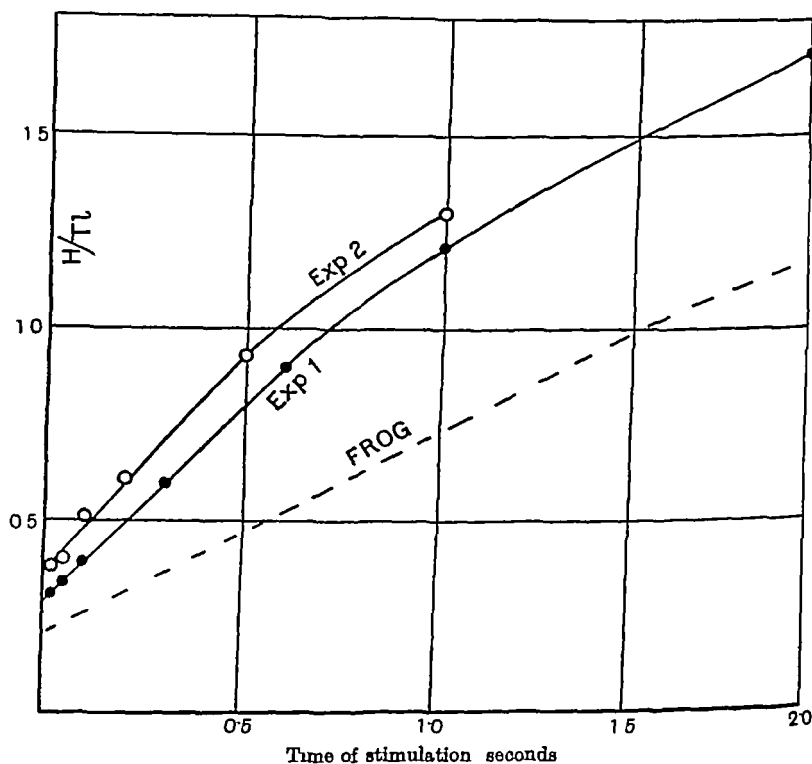


Fig. 1

within the range 0.1–0.5. It is obvious that we are dealing, in dogfish muscles as in all others investigated, with what is fundamentally the same mechanism, working with approximately the same “efficiency”.

As regards the rate of increase of  $H/Tl$  with duration of stimulus, a comparison with the curves marked 10° and 15° C in Fig 4 of the paper by Hartree and Hill(2) will show a fairly close similarity between the present experiments on dogfish and those on frog. In Fig 1 for comparison is given a curve roughly interpolated for 12° C (the tempera

ture of the present experiments) from the data of Hartree and Hill. The curve for dogfish rises initially nearly twice as quickly as that for frog, a fact to be associated with the great speed of movement of this animal's muscles, which, when in good condition, attain their maximum tension even at 12° C within a few hundredths of a second of stimulation. The rate of rise is many times as great as for the tortoise. Quickness of contraction and relaxation appears to be associated of necessity with wastefulness in maintaining a tension. This wastefulness causes the rapid onset of fatigue, and the tailing off of the tension during the longer stimuli. In the experiments of Hartree and Hill on frog there is no important tendency for the  $H/Tl$ -duration curves to fall away from linearity: in the present experiments a noticeable falling away occurs after about 0.5 sec. It would seem probable that in the completely fresh muscle, if the technique allowed it to be studied, the relation would really be linear, and that it would tend to fall away from linearity only when oncoming fatigue caused a lengthening out of the individual twitch, and a more ready and economical summation.

#### SUMMARY

- 1 The heat-production has been studied in the muscles of the lower jaw of the dogfish, and curves made relating  $H/Tl$  to duration of stimulus.
- 2 An optimal length (giving a minimum value of  $H/Tl$ ) exists as for other muscles.
- 3 The absolute value of  $H/Tl$  for short stimuli lies within the usual range found for other muscles.
- 4 The rate of rise of  $H/Tl$  with duration of stimulus is large, being about twice as great as in the frog and many times as great as in the tortoise. This is to be associated with the rapidity of response of these muscles, and in consequence of it the onset of fatigue during a prolonged stimulus is rapid.

My sincere thanks are due to Dr E. J. Allen and to Mr C. F. A. Pantin of the Marine Biological Laboratory, for their hospitality and help. I am indebted to Mr A. C. Downing and Mr J. L. Parkinson for their assistance in these experiments.

The expenses of this research have been borne in part by a grant from the Government Grants Committee of the Royal Society.

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# THE ANTAGONISM OF ACETYL CHOLINE BY METHYLENE BLUE By R P COOK

(From the Department of Pharmacology, University College, London)

STRAUB(1), after working with muscarine and atropine on the hearts of *Aplysia* and the frog, concluded that their action depended on the concentration of the drug around the cells and that the entrance of the drugs into the cells actually antagonised the action, which they produced on the surface. Recently Clark(2) showed that the amount of action produced by acetyl choline did not appear to be proportional to the amount of drug taken up by the cell and concluded that two independent processes occurred when acetyl choline acted on the frog's heart, viz (a) the action of the drug on the cell, probably a surface action, and (b) the entrance of the drug into the cell.

This relation can best be studied with a dye, methylene blue being a very suitable agent because in moderate concentrations it paralyses the vagus (Heymans and Maigre(3), (4)) without producing any injury to the heart cells. In the experiments described below isolated hearts of *Rana temporaria* were used. They were prepared with a Straub cannula containing 1 c.c. of Ringer's solution. The Ringer's solution was of the same composition as that used by Clark(2). The isotonic movements of the ventricle were measured.

Methylene blue was found to antagonise the action of acetyl choline

TABLE I Antagonism of acetyl choline by methylene blue.

The table shows the percentage reduction in response of the heart produced by varying the concentration of acetyl choline

Molar concentrations of methylene blue	Molar concentrations of acetyl choline							
	$9 \times 10^{-8}$	$2.6 \times 10^{-7}$	$8 \times 10^{-7}$	$2.4 \times 10^{-6}$	$7 \times 10^{-6}$	$2.2 \times 10^{-5}$	$6 \times 10^{-5}$	$2 \times 10^{-4}$
Nil	30	47	67	70	85	—	—	—
$6 \times 10^{-7}$	32	43	60	70	—	—	—	—
$3 \times 10^{-6}$	15	20	30	63	71	—	—	—
$6 \times 10^{-6}$	—	—	9	21	56	71	94	—
$3 \times 10^{-5}$	—	—	—	14	23	28	50	67

on the frog's heart in a manner similar to atropine. Table I gives a series of measurements of the effects produced by acetyl choline in the presence

of varying amounts of methylene blue, and Fig 1 gives a graphical interpretation of these results

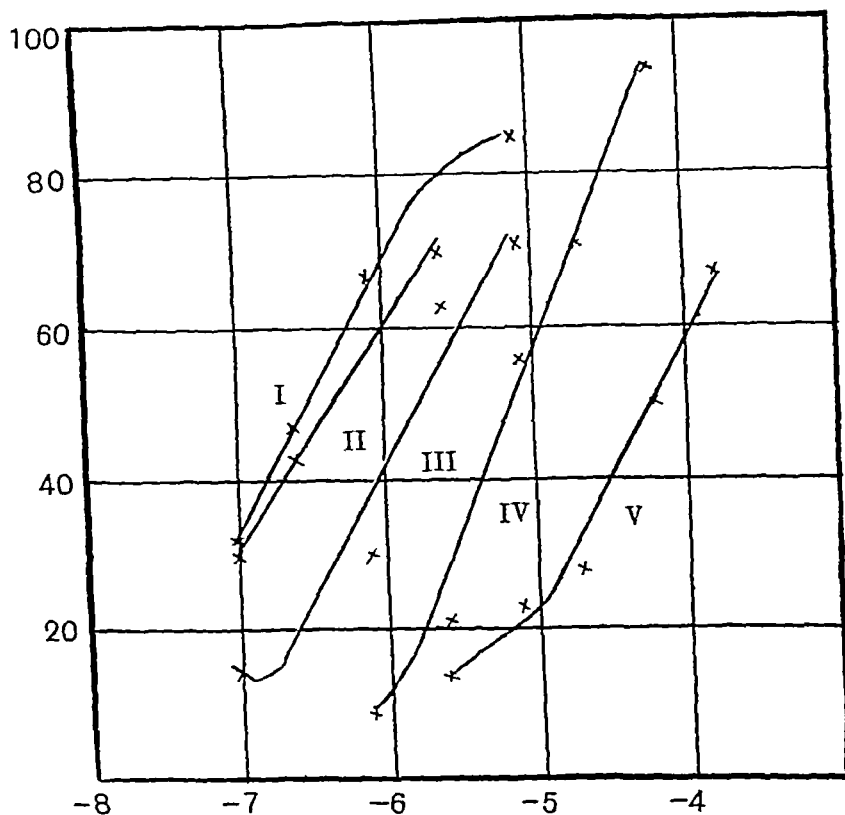


Fig 1 Antagonism of acetyl choline by methylene blue

Action of acetyl choline (I) in absence of methylene blue (II) in presence of methylene blue  $6 \times 10^{-7}$  molar, (III) in presence of methylene blue  $3 \times 10^{-6}$  molar, (IV) in presence of methylene blue  $6 \times 10^{-6}$  molar (V) in presence of methylene blue  $3 \times 10^{-5}$  molar

Ordinate percentage reduction in response of heart.

Abscissa log molar concentration of acetyl choline

The quantities of acetyl choline which produce a 50 p c reduction in the response of the heart in the presence of methylene blue are shown in Fig 2 Clark(5) showed that, when atropine and acetyl choline act on the frog's heart, equal effects are produced as long as the ratio

$$\frac{\text{concentration of acetyl choline}}{\text{concentration of atropine}}$$

remains constant The curve in Fig 2 actually follows the relation that constancy of the ratio

$$\frac{(\text{concentration of acetyl choline})}{(\text{concentration of methylene blue})^{1.25}}$$

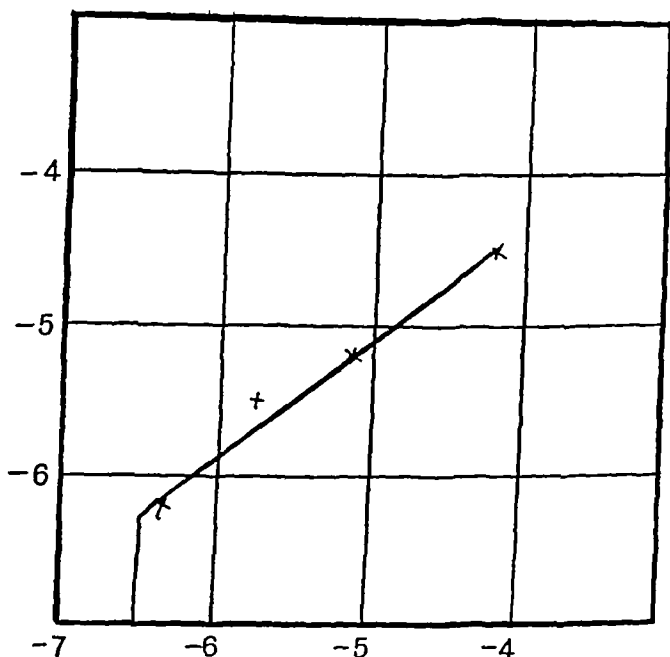


Fig 2 Quantities of acetyl choline necessary to produce a 50 p c reduction in the heart's response.

Ordinate log molar concentration of methylene blue

Abcissa log molar concentration of acetyl choline

leads to constant results The experiments, however, were not sufficiently extensive to justify any definite conclusion as to whether in the formula

$$\frac{(\text{conc Ac Ch})}{(\text{conc M.B})^n} = \text{constant},$$

$n$  is unity or more than unity

The frog's heart is stained a deep blue with the concentrations of dye used in these experiments and it is not possible to wash out a noticeable quantity of the dye with frequent changes of Ringer in several hours When the dyed hearts were examined microscopically the dye was found to be present in the interior of the muscle cells and also the nerve endings were deeply stained This condition remained unchanged

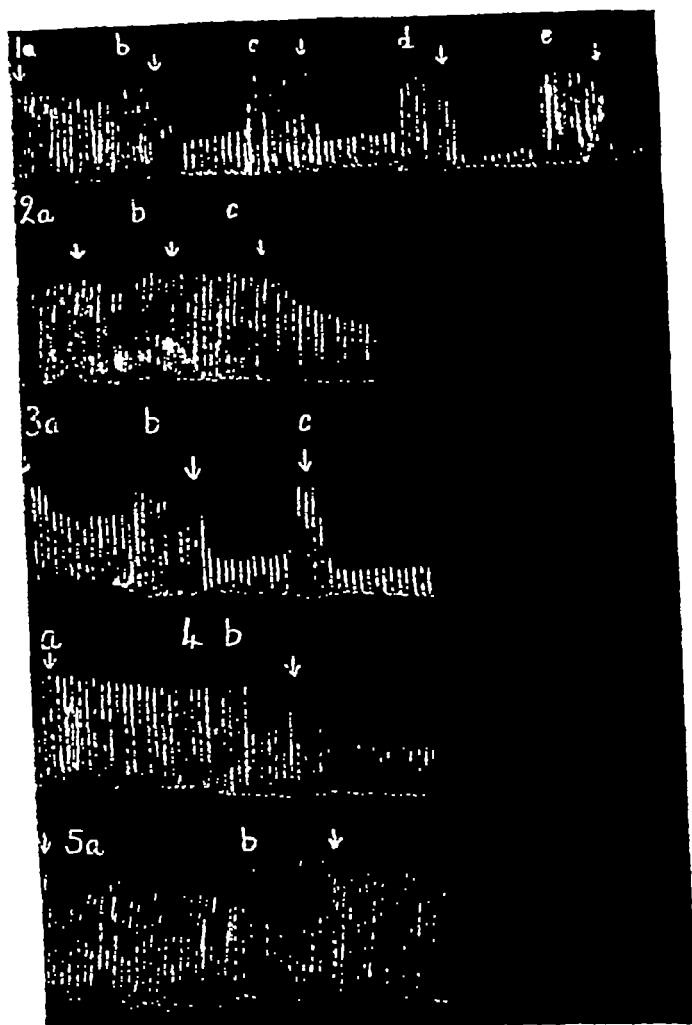


Fig 3 Heart under artificial rhythm of 20 shocks per min.

- (1) Action of acetyl choline in absence of methylene blue Molar concentrations (a)  $5 \times 10^{-8}$  (b)  $5 \times 10^{-7}$  (c)  $5 \times 10^{-6}$  (d)  $5 \times 10^{-5}$  (e)  $5 \times 10^{-4}$
- (2) Methylene blue  $3 \times 10^{-5}$  molar and acetyl choline (a)  $5 \times 10^{-8}$ , (b)  $5 \times 10^{-6}$  (c)  $5 \times 10^{-4}$  molar
- (3) Methylene blue washed out with Ringer's solution for the following times (a) 1 min. (b) 7 mins. (c) 17 mins. acetyl choline  $5 \times 10^{-6}$  molar added in each case
- (4) Methylene blue  $3 \times 10^{-5}$  molar (following 3) and acetyl choline (a)  $5 \times 10^{-8}$  (b)  $5 \times 10^{-4}$  molar
- (5) Heart washed out after 4 and then methylene blue  $3 \times 10^{-5}$  added (a) acetyl choline  $5 \times 10^{-8}$  molar added at once after methylene blue and (b) the same 4 mins later



even after frequent washings with Ringer's solution. The staining effects produced by the dye were therefore practically irreversible, but in spite of this fact, the action of methylene blue in antagonising acetyl choline was found to be rapidly and completely reversible.

Fig 3 shows the action of acetyl choline on a frog's heart after methylene blue had been alternately introduced and removed. The heart during the greater part of the experiment was dyed deeply blue.

These results show that the action of methylene blue in antagonising acetyl choline is independent of the quantity of methylene blue taken up by the muscle cells and nerves, and appears to depend entirely on the concentration of the dye in the fluid around the cells. This suggests that

TABLE II.

Rate of action of methylene blue		Rate of wash out of methylene blue	
Time (in mins.) since introduction of drug	Percentage re- duction produced by $5 \times 10^{-6}$ molar acetyl choline	Time (in mins.) since removal of drug	Percentage re- duction produced by $5 \times 10^{-6}$ molar acetyl choline
0	83	1	36
2	43	3	43
5	19	5	53
10	13	7	64
—	—	9	71
—	—	13	73
—	—	17	80

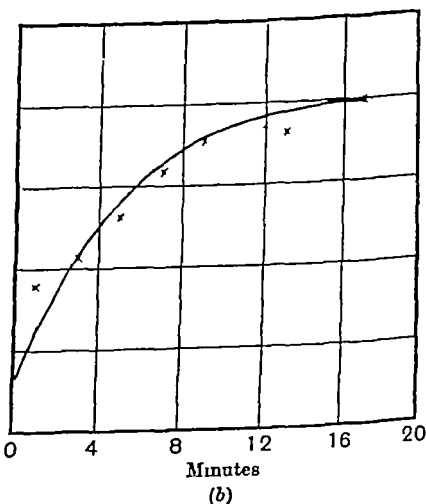
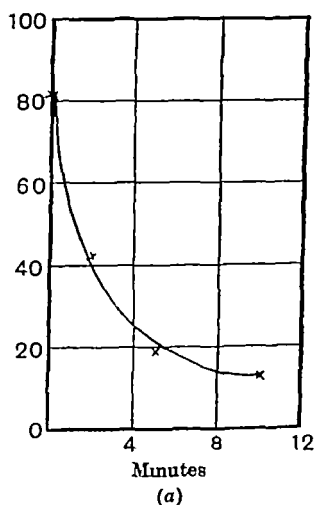


Fig 4 (a) Rate of action of methylene blue (b) rate of wash out of methylene blue.  
Ordinate percentage reduction in response of heart when  $5 \times 10^{-6}$  molar acetyl choline was added

antagonism is due to the methylene blue producing some freely reversible surface action on the cells

Table II and Fig 4 show the results of experiments made to measure the rate of action and the rate of wash-out of the methylene blue as indicated by the response to acetyl choline. These figures show that methylene blue produces half its final action in about 2 minutes and that the abolition of action on removal of the dye proceeds at a similar rate.

Accurate measurements of the rate of adsorption of methylene blue by the heart were very difficult to obtain. 1 c.c. of fluid containing methylene blue was introduced into the heart, the amount of dye remaining in the fluid was determined colorimetrically, and the amount of dye taken up by the heart was thus estimated. The results showed that the concentration of dye in the heart cells was from 20 to 200 times the concentration in the fluid around the cells. The adsorption of the dye proceeded slowly but was nearly complete at the end of an hour. The wash-out of the dye, if it occurred at all, proceeded too slowly to be measurable.

#### CONCLUSIONS

(1) Methylene blue antagonises the action of acetyl choline, and equal effects are produced by acetyl choline in the presence of methylene blue when the ratio  $\frac{\text{concentration of acetyl choline}}{\text{concentration of methylene blue}}$  remains constant. The value of  $n$  is either unity or slightly more than unity.

(2) The heart cells adsorb methylene blue slowly and the action is practically irreversible.

(3) The action of methylene blue in antagonising acetyl choline is produced rapidly and is removed equally rapidly by washing out the methylene blue. A heart can regain its full sensitivity to acetyl choline although deeply stained with methylene blue.

(4) This antagonism therefore is independent of the entrance of the dye into the nerve and muscle cells, the dye appears to produce its antagonism to acetyl choline by a freely reversible action on the surface of the cells.

I should like to take this opportunity of thanking Prof. A. J. Clark for the suggestions and help he has given me in this research.

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tone in these vessels at death. But during a perfusion experiment it gradually disappears, and it is essentially with the prevention of this loss of tone that we are concerned. It may be that changes in the tissues are sufficient to account for it. Investigation showed that Ringer's fluid perfused through the hind limbs of the cat would not maintain this tone, and, even if a response to histamine could be obtained when the preparation was first set up, it disappeared in a minute or two. It was found that the fluid passing through the tissues underwent considerable change, a solution which entered with  $pH\ 7.4$  showing a reaction of  $pH\ 7.0$  or even less on emerging from the veins. Since the presence of lactic acid in the perfusate was demonstrated by Hopkins' test, the formation of lactic acid and other metabolites with a considerable change in the hydrogen-ion concentration was regarded as a possible factor concerned in the loss of tone.

Having regard to this, perfusions were performed using alkaline Ringer's solution to counteract the effect of the acids produced. Their formation was further retarded, as mentioned above, by preliminary narcotisation of the animal and the injection of 1-2 c.c. of  $N/100\ NaOH$  or saturated  $NaHCO_3$ , spread over two or three hours to ensure the taking up of the alkali by the tissues. The perfusing fluid had the composition  $NaCl$ , 0.9 p.c.,  $KCl$ , 0.42 p.c.,  $CaCl_2$ , 0.24 p.c. and the reaction was adjusted by adding  $M/5\ Na_2HPO_4$  solution in the proportion of 40 c.c. to a litre of Ringer's solution and then bringing to the required reaction by  $M/5\ NaH_2PO_4$  solution, the quantities of which had previously been determined. The method of perfusion is described elsewhere<sup>(11)</sup>. If these precautions were taken, then no difficulty was experienced in demonstrating the dilator action of histamine several hours after the beginning of an experiment where the perfusing fluid has consisted merely of aerated Ringer's solution. Fluid adjusted to  $pH\ 7.6-7.7$  has been found to give the best results, and with this reaction the emerging fluid showed a reaction of  $pH\ 7.0-7.2$ , so that the reaction of solution through the capillaries must have been in the region of 7.4, that is, close to what we may imagine the normal value. Fig. 1 shows the response to histamine in a perfusion which had continued for more than an hour. Here the increase in outflow also is shown by connecting the outflow cannula from the inferior vena cava by means of a T-tube to a manometer and piston recorder.

A slight change in the perfusing fluid towards acidity is sufficient to cause the disappearance of the response, and this is clearly shown in Fig. 2 where histamine was dilating when the perfusing fluid was at

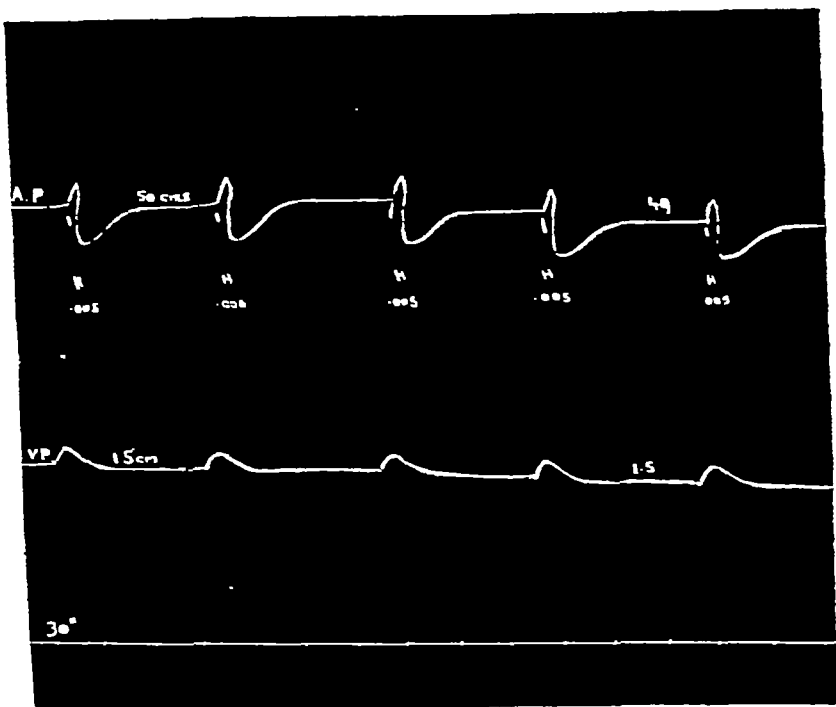


Fig. 1 Showing the dilator action of histamine one hour and a half after the commencement of perfusion. Upper tracing shows pressure of fluid entering aorta. Lower tracing indicates rate of flow from inferior vena cava pressure in cm.  $H_2O$ . The initial rise in the curve is due to the injection.

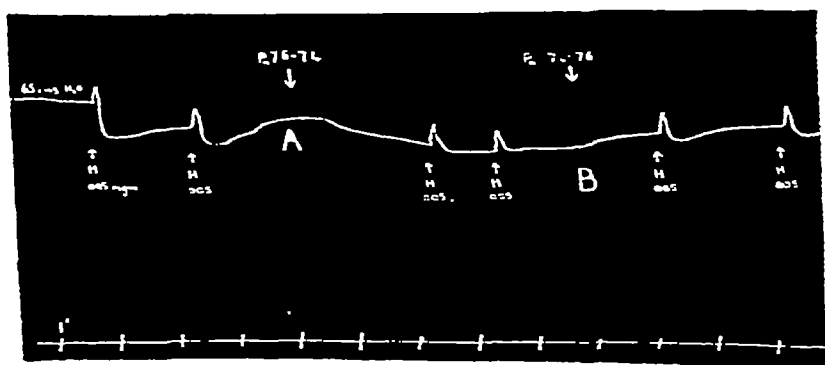


Fig. 2 Dilator action of histamine perfusing fluid pH 7.6. At A the solution is changed for one of pH 7.4. There is diminution in peripheral resistance due to loss of capillary tone and disappearance of dilator action. Original fluid restored at B with increase of tone and reappearance of histamine reaction.



Herein appears to lie the importance of previous treatment of the animal so as to delay post-mortem changes, since it makes it possible to perfuse the capillaries with a solution which will just bring back this tone without causing any appreciable effect on the arteries

The difference in this method and the precautions previously found necessary may then be briefly emphasised. No hormonal substances have been introduced, neither has the viscosity nor the oxygenation of the perfusing fluid been specially considered. The results may be explained as due to the prevention or hindering of the action of lactic and possibly carbonic acid.

It is interesting to observe that the capillaries of an animal which has died of asphyxic shock retain their sensitivity to histamine if perfused with an alkaline Ringer's solution of pH 7.6. This may readily be considered to be due to the alkalosis which results from the over-ventilation and the general slowing of metabolic processes caused by the shock. That such slowing does occur is suggested by the fact that in persons dying from hæmorrhage the onset of rigor mortis is delayed.

The third possibility, that the capillaries in some circumstances may not be able to show this response because of intense arterial constriction, has a certain amount of evidence in its support. It has been noted that immediately after death there is intense constriction of vessels. If arteries be isolated this constriction may, according to McWilliam, last for several days.

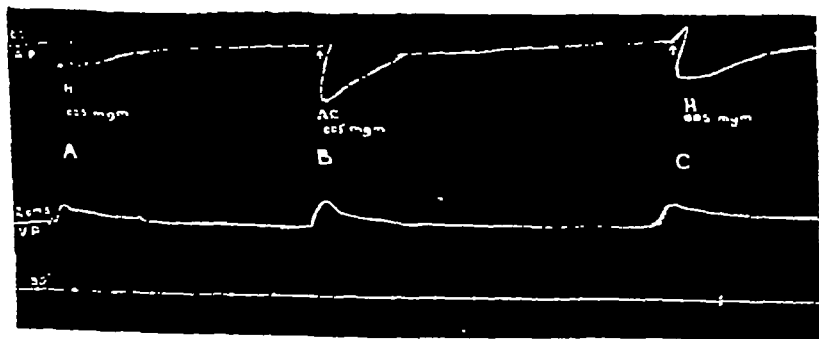


Fig 4. Histamine action increased after arterial dilatation with acetyl choline. At A 0.05 mgm histamine. Later at C the reaction has been increased following the dilatation due to acetyl choline at B.

In the present experiments it has been noticed not infrequently at the beginning of a perfusion experiment or later, if a very alkaline

solution is being perfused, that no dilator effect of histamine can be obtained unless an arterial dilator has been previously injected. The arterial dilator may simply cause the histamine response to be greater (Fig 4). The dilators used have been acetyl choline, small amounts of lactic acid and sodium nitrite. The effect of these arterio-dilators has not been to reduce the peripheral resistance permanently but only temporarily. The capillaries have then been more readily dilated by histamine, a result which we may presume has been brought about by a more adequate neutralisation and flushing of these vessels during the temporary arterial dilatation.

*Discussion* The above results show clearly that it is possible by simply paying attention to the acid-base equilibrium of the tissues and the perfusion fluids, to maintain, in isolated tissues, a condition of tone which is relaxed by histamine. In view of the known action of this drug it may be presumed that the tone concerned is that of the capillaries. It is, however, significant to observe that the point at which tone is maintained is appreciably on the alkaline side of absolute neutrality. This point we purpose describing as the point of "functional neutrality" and is, we believe, approximately at the hydrogen-ion concentration of the blood.

It has also been seen that in studying the reactions of isolated vessels it is important to consider not only the reaction of the perfusion fluid but also the rate at which acid products are formed by the tissues concerned. We believe that many of the diverse results which have been obtained in relation to the investigation of drugs have been complicated by this factor and it seems most probable that the selective action of certain drugs may be ultimately dependent on these facts. Since we know from the work of Hoskins, Gunning and Berry<sup>(12)</sup>, Hartman and Fraser<sup>(13)</sup>, that adrenaline in a given concentration may dilate the vessels of muscles and constrict those of the skin, and since we may presume that acid is more rapidly produced in muscles than in the skin, it seems reasonable to suppose that this apparently differential action of adrenaline is due to differences in the hydrogen-ion concentration of the regions concerned.

It is obvious, too, that where a closed system has been used in perfusion experiments or in the heart-lung preparation there must be a considerable alteration in the condition of the experiments as they proceed unless adequate steps are taken to buffer the fluids concerned.

An appreciation of these points throws considerable light on the results of Dale and his co-workers on this subject. It is evident that

the importance of adequate oxygenation lies not only in the supply of oxygen to maintain life, but is also closely related to the oxidation of lactic acid as it is formed. It is possible, too, that the addition of blood corpuscles to the perfusion fluid, which was emphasised by Dale and Richards, not only increases the viscosity of the fluid but also increases its buffering.

To what extent the general results given in this paper are related to the nervous and hormonal control of the capillaries will be made the subject of further investigation but meantime it appears evident that there exists in the variability of the acid-base equilibrium of the capillaries a mechanism which can regulate the blood supply of the tissues concerned according to their activity.

#### SUMMARY

Experiments are described which show that the maintenance of capillary tone as demonstrated by the response of these vessels to histamine is under the control of a simple acid-base equilibrium.

The expenses of this investigation were in part defrayed from a grant from the Government Grants Committee of the Royal Society.

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THE RÔLE OF THE PHOSPHATES IN CARBOHYDRATE  
METABOLISM IN SKELETAL MUSCLE Part II A  
comparison between the muscles of normal, fatigued  
and depancreatized animals

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IN our earlier paper<sup>(1)</sup> dealing with this subject the muscles of normal animals were compared with those from animals subjected to the action of adrenaline, insulin, and adrenaline plus insulin, attention being directed specially to the synthesis of the hexosediphosphate. The amount of the pre-existing and of the added precursors of the ester (glycogen and phosphoric acid) which can be synthesised in the presence of the fluoride anion by minced normal muscle may be taken as a standard of efficiency, so far as one link in the chain of the carbohydrate transformations in the muscle is concerned. Under normal conditions within the muscle the bound phosphate remains at a fairly constant level, although the ester may be readily broken down by fatigue (Embsen and Jost<sup>(2)</sup>, Andrews<sup>(3)</sup>), or in the condition of rigor mortis (Deuticke<sup>(4)</sup>). This constancy is undoubtedly due to the fact that the compound appears as an intermediary in the transformation of glycogen to lactic acid, and as this process is a reversible one, its concentration is determined by the balance struck between the hydrolytic and the synthetic processes. While the glycogen content of skeletal muscle may show wide variations even in well-fed cats, say from 0.5 to over 1 p.c., the amounts of the bound phosphate in the normal muscle, as a rule only vary within quite a narrow range, say from 0.18 up to 0.22 p.c. Under the action of insulin there may be, and indeed there usually is, an increase in the amount of bound phosphate, but even were such an increase not detectable on analysis, one cannot conclude that the process of esterification has not been aided by this hormone as the transformation rate through the later stages leading to the partial combustion of the glucose may also be quickened. In order to detect whether the esterification process itself has been altered during some disorder of carbohydrate metabolism, the

method of checking both hydrolysis of the ester and also oxidation by the use of the fluoride anion has been made use of. Embden and his school have drawn attention to the marked synthetic effect of this anion, giving rise to a very great decrease in the concentration of the free inorganic phosphates. Not only is such the case but the normal muscle of the cat can synthesise in addition large quantities of added phosphate, if a sufficiency of glycogen be supplied. One may therefore determine the optimal capacity for esterification of the muscles of the normal animal, and if this be found to be fairly constant under certain fixed conditions, it may be used as a standard with which the muscles of animals suffering from various disturbances in carbohydrate metabolism may be compared. If the hexosediphosphate be an essential link in the chain of carbohydrate transformations in the active muscle, it is necessary to gain as much knowledge as possible of the conditions under which the combination between glycogen (the precursor of the hexose) and phosphate is most readily brought about, and also of the conditions which interfere with the esterification. The fact that the synthetic process under fluoride takes place with a concomitant disappearance of glycogen and a check in lactic acid formation shows that one stage, and one alone, in the cycle is being dealt with. This synthetic process is seriously interfered with if there be traces of metallic impurity (copper) in the distilled water, and in our opinion it is advisable to use tap-water to make up all the solutions with which the minced muscle is brought into contact.

Throughout the course of the investigation the normal cat's muscle was analysed frequently and the effects of variations in the nutritive condition, as well as the effects of slight and prolonged stimulation were also studied in order that a knowledge of the specific effects of depancreatisation might be compared with those resulting from a simple lowering in the muscle glycogen store. The differences between the muscles of the normal and depancreatised animals were studied in the following ways:

I The amounts of phosphoric acid, glycogen, and lactic acid were determined in the muscle immediately after removal, the so-called "existing condition."

II The "breakdown process," when the minced muscle was kept in 2 p.c. bicarbonate solution at 45° C. for two hours, was investigated.

III The "synthetic process," as it occurs when the fluoride acts upon the intrinsic constituents, without further addition of the components of the ester, and

IV The synthetic action on the pre existing plus added precursors of the ester (glycogen plus phosphate) were also studied

In addition to analyses of the free phosphoric acid, glycogen and lactic acid, under these various conditions, the blood sugar and the liver glycogen were also determined whenever necessary. The methods employed for the analyses were those described in the earlier paper. The pancreas was removed in its entirety by one of us (T H M) in more than twenty animals in the following way. The glandular substance was cauterised lightly in the first place and, after tying the ducts, the gland substance was broken down carefully with small portions of sterile gauze and removed, leaving the duodenal circulation as far as possible intact. After removal, any small particles of gland substance on the duodenal wall and along the course of the vessels were carefully cauterised, and finally, when all bleeding had ceased, the duodenum was carefully wrapped round with the omentum which was held in position by a few catgut stitches. The animals recovered perfectly and immediately after the operation moved about freely. They were kept warm and given hot milk and water at the outset, and whole milk during the rest of the period. There were no signs of sickness. The muscles were examined after varying periods, from one to ten days after the operation.

The degree of disturbance produced in the muscle did not appear to bear a quantitative relationship to the interval allowed to elapse between the operation and the removal of the muscle, nor to the degree of hyperglycæmia produced. In almost every case, even after a short period, the liver glycogen had fallen to a low level (0.1 p.c. and under) while the muscle glycogen was much more resistant, rarely falling below 0.2 p.c., the average value in normal animals being about 0.6 to 0.7 p.c.

To avoid repetition, the results obtained for the normal well-fed animal's muscles will in each case be compared with those found after starvation, stimulation and pancreatectomy. The average results obtained for the normal well-fed animal differ in certain respects from those given in the earlier paper, and since these were derived from a much larger series of experiments which were carried out during the course of the work embodied in this paper, they are more suitable for comparison.

#### I *The existing condition in the muscle (adductors)*

1 Normal well-fed animal. The liver glycogen varied usually between 3-6 p.c., the blood sugar (under light ether anaesthesia) averaged 0.15 p.c.

## Analyses of muscle constituents

(a) The glycogen percentage ranged from 0.5 to 1 (average 0.64 p.c.), the store rarely falling to the lower level even when the liver glycogen in the less well-fed animals had fallen to 1 p.c. or under

(b) The lactic acid varied from 0.075 p.c. to 0.25 p.c., the average being 0.117 p.c. The higher values were obtained with very excitable muscle which also showed more active synthesis under fluoride

(c) The free phosphate concentration is the most constant and appears to be independent of the nutritive condition of the animal. Per gramme muscle the average content was 2.94 mg  $H_3PO_4$ , with a range from 2.59 to 3.38

The value of the bound phosphate will be given under the breakdown process

The averages referred to above were obtained from a series of twenty normal animals

Two types of normal animals may be compared, both well-fed but differing greatly in their liver glycogen.

TABLE I.

	I	II
	p.c.	p.c.
Liver glycogen	5.240	1.00
Muscle glycogen	0.530	0.48
Free phosphate ( $H_3PO_4$ )	0.260	0.31
Lactic acid	0.085	0.24

A high muscle glycogen, low free phosphate, and low lactic acid often are indications of less excitable muscle. The bound phosphate in I was also much higher (0.222) than in II (0.187). Evidently the former offered greater resistance to the natural breakdown process which accompanies the mincing of the cooled muscle.

## 2 The starved animal

(a) The muscle glycogen percentage after 3-4 days' starvation never fell below 0.2 and after a shorter starvation period (2 days) it remained about 0.5

(b) The lactic acid average percentage was slightly over that found in the well-fed animal, namely 0.15

(c) The free phosphate average value was the same in both.

3 The effects of stimulation of the muscle on the existing condition will be dealt with more fully in another communication, but an example of the effect of prolonged stimulation may be given. Table II gives the values obtained for the gastrocnemius muscle where the sciatic nerve on

one side was stimulated for two hours by slow interrupted faradic stimulation

TABLE II.

	Stimulated p c.	Unstimulated p c.
Glycogen	0 024	0 541
Free phosphate ( $H_2PO_4$ )	0 357	0 282
Lactic acid	0 221	0 057

The rise in the free phosphate was naturally due to the breakdown of hexosediphosphate. When the stimulation was for a much shorter period, 10–15 minutes, the glycogen always fell slightly with an accompanying slight lactic acid rise, while the free phosphate remained practically unaltered.

#### 4 The depancreatized animal

(a) Glycogen. The liver glycogen even after two days was usually brought down to a very low level (0 1 p c or under), while the muscle glycogen, although it might fall from 0 6 to 0 12 p c within two days, was usually maintained at a much higher percentage, for example 0 3, even after six days had elapsed since the removal of the pancreas and with a blood sugar of 0 42 p c.

(b) Lactic acid. As in normal muscle there is a wide range of variation, but in the depancreatized state the original value is often much lower than is ever found in the normal muscle, 0 05 p c and under. The exceptionally low values are found in cases where the bound phosphate is diminished, while the higher values (0 1 p c or slightly over) are met with in cases where, if any phosphate diminution has occurred, it has done so at the cost of the free form.

(c) Free phosphate. The average percentage is slightly higher than in normal muscle, namely 0 308 p c (range from 0 259 to 0 377).

## II The breakdown process

### 1 In the normal well-fed animal's muscles

(a) Glycogen practically disappeared entirely.

(b) Lactic acid reached on an average 0 5 to 0 6 p c although in some cases with an original high glycogen value, a greater production was met with. A lower maximal acid production (0 4) was met with in certain cases where the original lactic acid value was low, and the muscle fairly well stored with glycogen.

(c) Free phosphate. The increase in free phosphate produced by hydrolysis was derived from the originally bound form and amounted

on an average to 0.188 p.c. Of the total phosphate present in the normal muscle 36–40 p.c. was in the bound form

## 2 In the starved animal

(a) Glycogen disappeared, as always occurs during hydrolysis

(b) Lactic acid production reached as an average maximal value 0.5 to 0.6 p.c., which was in part derived from the original small glycogen store, and in part from the lactacidogen.

(c) The increase in the free phosphate was the same as in the well-fed animal, the hexosediphosphate thus being present in the same amount in both. An example of the breakdown process in the starved animal's muscle will show the nature of the changes produced

TABLE III

	H <sub>3</sub> PO <sub>4</sub> p.c.	Lactic acid p.c.	Glycogen p.c.
Original condition	0.313	0.139	0.234
After hydrolysis	0.540	0.612	—

During the hydrolysis, per gramme muscle, over 4 mg. lactic acid have appeared, 2 mg. of which could have been supplied by the glycogen and 2 mg. from the hexosediphosphate

3 The stimulated muscle After prolonged stimulation (2 hours), hydrolysis produced very little further change as the glycogen and the hexose diphosphate had both reached a low value. Thus in the case of the muscle referred to in Table II the lactic acid showed no further increase while the free phosphoric acid value rose from 0.357 to 0.422 p.c., corresponding to a lactacidogen phosphoric acid value of 0.065 p.c. When the stimulation was of very short duration (10–15 mins.) the existing condition of the muscle was found to be the same on the stimulated as on the unstimulated side, and therefore hydrolysis brought about the same changes in both sets of muscles. When stimulation was rather more prolonged the glycogen and the hexosediphosphate percentage in the muscle gradually fell and the lactic acid rose, so that on subsequent hydrolysis the lactic acid and free phosphoric rise showed a smaller increase than in the case of the normal rested muscle

4 After pancreatectomy The breakdown process showed certain variations which indicate that there are at least two different types of muscle met with after pancreatectomy. In these types both the breakdown and synthetic processes differ. The first type is met with in animals in very good condition, apart from the high blood sugar and low liver glycogen. There is no appearance, so far as the general behaviour and

muscular movements are concerned, of any departure from the normal. Three examples of the breakdown process in this type may be given. In (a) the muscles were examined 24 hours after removal of the pancreas, in (b) after 48 hours and in (c) after 75 hours

TABLE IV

	H <sub>2</sub> PO <sub>4</sub> p c	Lactic acid p c	Glycogen p c	Blood sugar p c
(a) Before hydrolysis	0.314	0.03	0.27	0.32
After hydrolysis	0.487	0.54	0.03	
(b) Before hydrolysis	0.308	0.03	0.44	0.47
After hydrolysis	0.490	0.66	0.04	
(c) Before hydrolysis	0.318	0.18	0.37	0.36
After hydrolysis	0.493	0.51	—	

In all the specimens the hexosediphosphate, as determined from the increase in free phosphate on hydrolysis, and the glycogen are below the normal. The increment in lactic acid is the same as would occur on hydrolysis of normal muscle.

The other type is mainly characterised by a small lactic acid increase during the breakdown process. The animals were more lethargic than those of the other type, one (C<sub>1</sub>) dying suddenly under ether just as the blood was being withdrawn.

Five examples of this type will be given.

TABLE V

	H <sub>2</sub> PO <sub>4</sub> p c	Lactic acid p c	Glycogen p c	Blood sugar p c	Time depan- creatised
(a) Before hydrolysis	0.297	0.09	0.565	0.27	48 hours
After hydrolysis	0.459	0.32	—		
(b) Before hydrolysis	0.377	0.09	0.379	0.29	4 days
After hydrolysis	0.401	0.27	—		
(c) Before hydrolysis	0.287	0.05	0.488	0.35	2½ "
After hydrolysis	0.455	0.17	—		
(d) Before hydrolysis	0.313	0.14	0.472	0.25	4 "
After hydrolysis	0.477	0.37	0.204		
(e) Before hydrolysis	0.335	0.15	0.482	0.38	2 "
After hydrolysis	0.471	0.35	0.208		

These five cases, and there were many more of the same type, show the following peculiarities in the breakdown process. There is a distinct diminution in the amount of phosphate set free by hydrolysis and hence a lessened lactacidogen content. The increase in lactic acid is extremely small and can nearly, or in one case altogether, be accounted for by the hexosediphosphate breaking down with only a small portion derivable from the glycogen.

The original glycogen content of the muscle, even four days after removal of the pancreas, has remained at a fairly high level. As will be seen later, the synthetic processes in these muscles have been very seriously interfered with in most cases. The glycogen after hydrolysis was only determined in the last two, because unfortunately it was taken for granted in the others that it would have disappeared entirely but in the two mentioned there was still a fair amount of glycogen present so that the diminution in lactic acid production can be partly explained in this way.

### III. *The synthetic process under fluoride as it affects the intrinsic precursors of the ester*

1 In the normal animal in good condition. Three examples will be given, chosen because of the differences in the glycogen store in the muscle. The amounts of the free phosphate, lactic acid and glycogen before (A) and after (C) the action of the fluoride will be given.

TABLE VI.

	H <sub>2</sub> PO <sub>4</sub> p.c.	Lactic acid p.c.	Glycogen p.c.
(1) A	0.309	0.242	0.489
C	0.085	0.129	0.084
(2) A	0.296	0.252	0.711
C	0.051	0.191	0.356
(3) A	0.281	0.145	1.300
C	0.040	—	1.000

Per gramme muscle in (1) 2.24 mg have been synthesised out of 3.09 with a consumption of 4.05 mg glycogen along with a decrease of 1.13 mg lactic acid. in (2) 2.45 mg out of 2.96 with a consumption of 3.55 mg glycogen and a decrease of 0.61 mg lactic acid. in (3) 2.41 mg have been synthesised out of 2.81 and 3 mg glycogen have disappeared. The lactic acid in this muscle was not determined after synthesis. Of the intrinsic free phosphate, between 72.5 and 85.7 p.c. has been synthesised and during this synthesis lactic acid formation is not only checked but there is also a disappearance of the acid while an amount of glycogen has been consumed which is more than sufficient to supply the carbohydrate component of the ester formed.

The best synthesis has occurred when the glycogen consumption has closely followed in amount the removal of the free phosphate.

The effect of impoverishment of the muscle glycogen store on the synthetic process will now be referred to.



## 2 After starvation for four days (two examples)

TABLE VII.

	$H_3PO_4$ p c.	Lactic acid p c.	Glycogen p c.
(1) A	0 293	0 209	0 486
C	0 058	0 190	0 367
(2) A	0 313	Lost	0 234
C	0 081	0 139	0 130

In (1) the muscle glycogen store was practically the same as in one of the well-fed animals

In (1) 2 35 mg out of 2 93 and in (2) 2 32 mg out of 3 13 were synthesised by 1 gm muscle with a consumption only of 1 19 mg glycogen in (1) and of 1 04 mg in (2)

Less glycogen has been consumed than would be required for synthesis of the hexosediphosphate. From some experiments dealing with the time factor in this synthetic process there was evidence of a synthesis of glycogen under the action of fluoride. It is probable therefore that, for the synthesis of the hexosediphosphate, glycogen formation during the esterification process is required. As has been shown by Embden, the sugars, as such, cannot replace glycogen in facilitating synthesis.

3 The effect of stimulation of the muscle on the synthetic process. If the stimulation is brief and does not result in a distinct decrease in the muscle glycogen store, the synthesis of the intrinsic precursors is as good as in the unstimulated muscle. Three examples of the effect of stimulation will be given.

TABLE VIII

	$H_3PO_4$ p c.	Lactic acid p c.	Glycogen p c.
(a) A	0 278	0 159	1 220
C	0 023	—	0 980
25 minutes' direct stimulation of exposed muscle			
(b) A	0 279	0 330	0 152
C	0 093	—	0 033
35 minutes' stronger direct stimulation of exposed muscle			
(c) A	0 357	0 220	0 024
C	0 314	0 210	0 054
2 hours' stimulation of sciatic nerve until gastronemius showed marked fatigue			

With progressive diminution in the glycogen store the synthetic process deteriorates, but even when it is lowered to 0 152 p c. one gm muscle can still synthesise 1 86 mg out of 2 79 mg  $H_3PO_4$ . In order that this quantity of phosphate should be changed to the hexosediphosphate form, at least 1 52 mg glycogen would be required, and slightly less than this, 1 19 mg have been used up. In the first example

the glycogen store in the muscle at the close of stimulation was very high and the synthesis was extremely good, 2.55 mg out of 2.78 with a consumption of 2.4 mg glycogen, while 2.09 mg would have, theoretically, been sufficient

In the last example where fatigue had set in after the prolonged stimulation, the muscle glycogen store was practically exhausted and after the synthesis the amount had apparently risen slightly, although 0.43 mg  $H_3PO_4$  had been synthesised out of 3.57. The glycogen values are however too low for accurate estimation. In all three cases the amount of glycogen which disappeared during the synthesis of the ester was small, and in the last two examples carbohydrate other than the original glycogen must have been used for the synthesis. It certainly appears as if the synthetic process is governed not only by the amount of the components of the ester available but also, in accordance with the view of Embden, by the effect produced on the enzymatic process by changes in the colloidal intrafibrillar mechanism

4 The effect of pancreatectomy on the synthesis of the intrinsic precursors of the ester. The effect was, in general, a diminution in the synthetic power. This diminution was not due to the exhaustion of the carbohydrate stores, as these were usually no more depleted than in the muscles of starved animals. The deterioration was most marked in the cases where the original lactic acid value was low and where, on hydrolysis, the total lactic acid production and the free phosphate increase were smaller than in the muscles of normal animals. Thus a muscle in which the breakdown process was interfered with showed a disturbance also in the synthetic process while in cases where the former differed but slightly from that of the normal animal the latter also approached the normal type. Two types of muscle in the depancreatized animal then are met with both as regards hydrolytic and synthetic changes. The synthetic changes in the intrinsic components will be given for the two types

The best type which approaches closely the normal (the breakdown process in which is given in Table IV) is shown in the following examples

TABLE IX.

	$H_3PO_4$ p.c.	Glycogen p.c.
(a) A	0.314	0.270
C	0.071	0.180
(b) A	0.308	0.441
C	0.056	0.098
(c) A	0.318	0.378
C	0.072	0.17

The synthesis is as good as in normal animals so far as capacity to synthesise the intrinsic free phosphate is concerned. In (b) and (c), but not in (a), the glycogen consumption is sufficient for esterification

Examples of the second type in which esterification as well as the breakdown process has been interfered with will now be given (the breakdown process is given in Table V)

TABLE X.

	$H_2PO_4$ p c.	Glycogen p c.
(a) A	0.297	0.565
C	0.139	0.109
(b) A	0.377	0.379
C	0.170	—
(c) A	0.287	0.488
C	0.284	0.342
(d) A	0.313	0.472
C	0.096	0.250
(e) A	0.335	0.482
C	0.147	0.300

The lactic acid production under the fluoride was checked in all the specimens. The phosphate synthesis was much worse than in the normal muscle. In (c) there was practically no synthesis, in (a), (b) and (e) between 52 and 56 p c., and in (d) 67 p c. of the intrinsic phosphate was synthesised. The glycogen consumption was sufficient to cover synthetic requirements. In (c) where synthesis was practically absent there must have been a serious disturbance in the muscle colloids associated with the enzymatic process, as there was a fairly high glycogen store in the muscle, a small amount being used up under the action of the fluoride without beneficial effect.

#### IV *The synthetic process after the addition of glycogen and phosphate*

From the earlier work it had been found that the normal muscle was capable of synthesising a larger quantity of phosphate than was originally present and that this synthesis was greatly improved by adding glycogen as well as phosphate.

In all cases examined the amount of the intrinsic plus added phosphate was approximately 13 mg (stated as  $H_3PO_4$ ) per gm muscle, sufficient glycogen being added to synthesise completely this quantity. As in all cases, the lactic acid production, even with the excess of glycogen and phosphate, was completely checked, it is not necessary to give the amounts of this acid before and after synthesis.

The glycogen (intrinsic and added) was almost completely removed

during the esterification. The amount of phosphate synthesised was always of the same order, the disappearance of the added free phosphate being most complete in those cases where the intrinsic phosphate and glycogen had been most efficiently esterified. The results may be given in condensed form

1 One gm minced muscle taken from an animal in good condition will synthesise on an average 11.5 mg out of 13 mg  $\text{H}_3\text{PO}_4$ , the phosphate being present in the form of  $\text{Na}_2\text{HPO}_4$ . The lowest values are slightly over 10 mg and the highest slightly over 12 mg

2 The average synthesis in the case of muscle from a starved animal is approximately 10 out of 13 mg. In both cases a sufficiency of glycogen disappears for the formation of the hexosediphosphate

3 After stimulation of the muscle for a short time (10–15 mins) the synthesis is quite as good as with the unstimulated muscle, in certain cases rather better, but, when the stimulation is prolonged and the intrinsic glycogen store brought down to a low level, the simple addition of glycogen along with the phosphate does not lead to the usual improved synthesis. In one case of extreme fatigue, only 1.72 mg were synthesised out of 13 mg, although the added glycogen had almost entirely disappeared under the action of the fluoride. Syntheses of 6–7 mg out of 13 mg were obtained after direct stimulation of the muscle for slightly over half an hour

4 After pancreatectomy. In two cases only did the synthesis of the intrinsic plus added phosphate approach that obtained in the case of the muscles of the normal animal. In each of these the synthesis was 10 out of 13 mg. In one of these, examined three days after the removal of the pancreas, the muscle glycogen was 0.378 p.c., the blood sugar 0.36 p.c., and the liver glycogen had almost entirely disappeared.

In the other, examined four days after removal of the pancreas, the muscle glycogen was much higher (0.693 p.c.), the blood sugar low (0.23 p.c.) and the liver glycogen 0.14 p.c.

The results obtained in thirteen depancreatized animals are given in the following table, the muscle and liver glycogen and the blood sugar as well as the phosphate synthesised being included

The animals which were in the best condition after removal of the pancreas synthesised from 6–8 mg out of 13 mg while those which were in poor condition and lethargic (therefore killed earlier) synthesised 3–5 mg out of 13. Usually the best syntheses occurred in cases where the muscle glycogen was about 0.4 p.c., although the percentage might be above that figure and esterification bad or below it and yet fairly good

TABLE XI

	Muscle glycogen p c.	Liver glycogen p c.	Blood sugar p c.	mg $H_3PO_4$ synthesised out of 13 mg per gm. muscle	Duration of depancreatization
(1)	0.28	0.08	0.20	3.94	28 hours
(2)	0.12	0.12	0.20	4.80	48 "
(3)	0.48	0.12	0.35	3.27	54 "
(4)	0.46	0.06	0.27	5.70	48 "
(5)	0.28	—	0.21	5.06	48 "
(6)	0.29	0.03	0.30	6.08	48 "
(7)	0.48	0.05	0.38	7.00	48 "
(8)	0.44	0.06	0.47	8.20	48 "
(9)	0.38	1.07	0.30	5.10	4 days
(10)	0.50	0.02	0.25	7.06	4 "
(11)	0.27	—	0.32	7.38	4 "
(12)	0.40	0.04	0.34	8.10	4 "
(13)	0.32	0.15	0.40	4.17	6 "

In none was the synthesis so good as with the muscles of the normal animal

In conclusion a brief reference may be made to a short series of experiments which was carried out to determine the changes which the free phosphate, glycogen and lactic acid undergo during the course of the fluoride action on the intrinsic, and the intrinsic plus added glycogen and phosphate. These experiments, dealing both with normal and diabetic animals, were carried out in the following way

The fluoride action on the minced muscle was checked in the usual way after  $\frac{1}{2}$  hour, 1 hour,  $1\frac{1}{2}$  hours and 3 hours, and after each of these intervals the free phosphate, lactic acid and glycogen were determined so that with the original (A) values the course of the reaction changes might be investigated

The results obtained in the case of normal cat's muscle are shown in Fig 1 (action on intrinsic components) and Fig 2 (action on intrinsic plus added components, 13 mg  $H_3PO_4$  and 13 mg glycogen per gm muscle)

The comparable results in the depancreatized animal are shown in Figs 3 and 4

Fig 1 shows the following

1 A rapid glycogen consumption during the first half hour, followed by a synthesis of glycogen and finally its disappearance

2 The synthesis of the phosphate has been completed within the first hour. The primary glycogen disappearance has occurred before the phosphate synthesis has been completed

3 A disappearance of lactic acid during the early part of the synthetic process

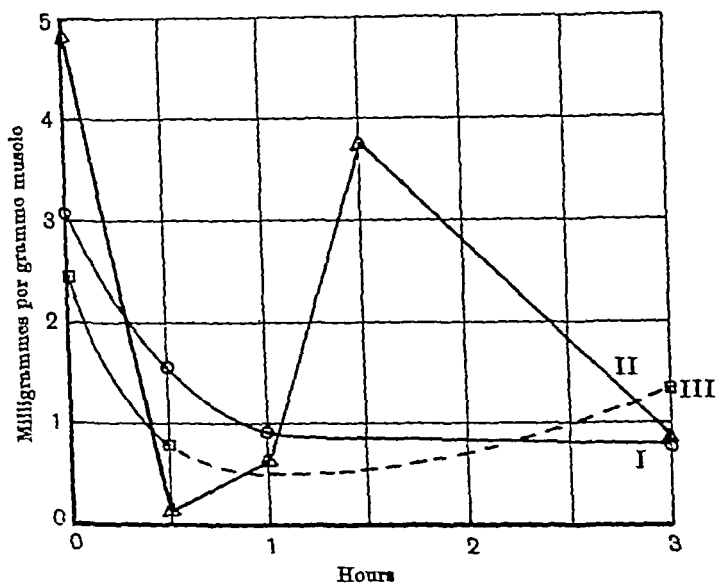


Fig 1 Normal cat's muscle. Synthesis under fluoride.  
 I = Phosphate (intrinsic), as  $H_2PO_4$  II = Glycogen (intrinsic)  
 III = Lactic acid.

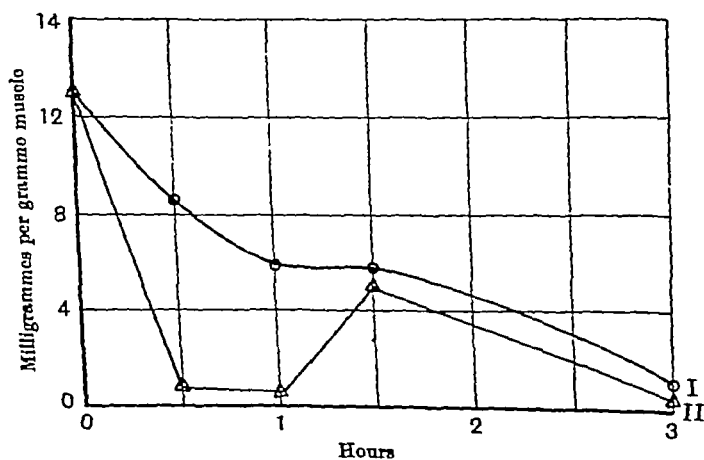


Fig 2. Normal cat's muscle. Synthesis under fluoride  
 I = Phosphate (intrinsic + added), as  $H_2PO_4$   
 II = Glycogen " "

Comparing this with the behaviour of muscle from the depancreatized animal, the following differences are to be seen (Fig 3)

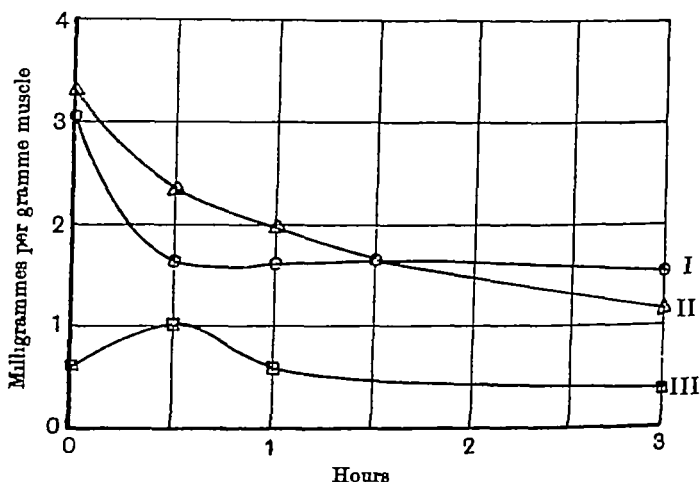


Fig 3 Depancreatized cat's muscle Synthesis under fluoride  
 I = Phosphate (intrinsic), as  $H_3PO_4$  II = Glycogen (intrinsic)  
 III = Lactic acid.

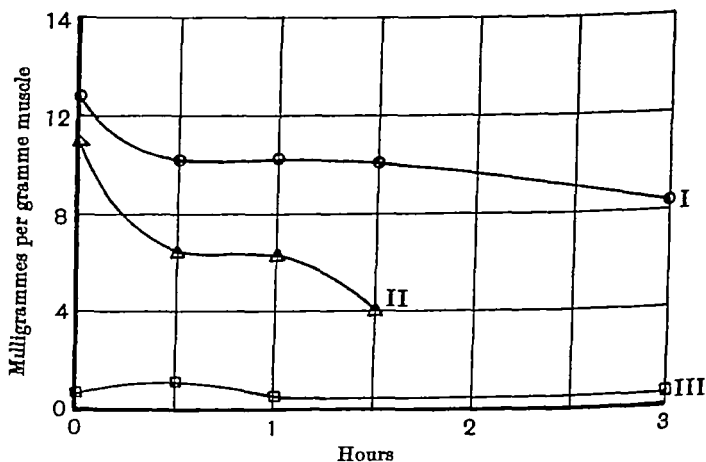


Fig 4 Depancreatized cat's muscle Synthesis under fluoride  
 I = Phosphate (intrinsic + added) as  $H_3PO_4$  II = Glycogen (intrinsic + added)  
 III = Lactic acid.

1 The glycogen consumption is more gradual and less complete  
 There is no evidence of a glycogen synthesis

2 The maximal phosphate synthesis has occurred early but to a lesser degree than in normal muscle. The lactic acid production is checked.

The syntheses of the intrinsic plus added components in the normal and depancreatized animals are shown in Fig. 2 and Fig. 4.

In the normal muscle the same rapid consumption of glycogen followed by a synthesis is to be seen, and the phosphate synthesis is not completed at the end of the  $1\frac{1}{2}$  hours period.

In the depancreatized animal the same type of reaction changes is seen with the added as with the intrinsic components alone, namely, a holding up of the glycogen, the consumption of which corresponds more closely to the amount required for the production of the hexosediphosphate than in the case of the normal muscle. There is also a great decrease in the amount of free phosphate synthesized.

#### DISCUSSION

From a study of the existing condition in muscle the carbohydrate mechanism appears to be a very stable one. The resistance offered to depletion of the glycogen store and the maintenance of the phosphate, organic and inorganic, at such a constant level indicate that reversible processes play important parts in the transformations.

The changes produced in the existing condition of the muscle by starvation and depancreatization are, in certain respects, very similar, but when the diabetic condition is accompanied by great weakness and lethargy the changes produced in the muscle by the hydrolytic action of weak bicarbonate solutions (the breakdown process) are very different. In the case of the starved animal the breakdown process is the normal one, while, in the diabetic, there is a much smaller production of lactic acid, the value after hydrolysis being as low as from 0.17 to 0.3 p.c. The difference in behaviour of these muscles is of interest, since, at the outset, both have a similar glycogen store. In some cases the amount of lactic acid produced in the depancreatized animal's muscle could be accounted for by the breakdown of hexosephosphate alone so that the glycogen must have been changed into some form other than lactic acid.

As regards the synthetic process, the muscles in which the hydrolytic change was abnormal showed a reduced power to synthesize both the intrinsic and the added precursors of the ester. That the failure to synthesize was not due to lack of the components is evident. The possible cause might be one or other of the following:



(a) An interference with the action of the esterifying enzyme or an alteration in the associated muscle colloids

(b) A change in the form of combination of the glycogen in the muscle

Undoubtedly the most marked interference with the synthetic mechanism is found in muscles which have been exhausted by prolonged stimulation or as a result of severe pancreatic diabetes especially in animals of poor condition, or in rigor mortis. In all of these conditions alterations in the muscle proteins are to be expected, so that Embden's hypothesis may furnish the correct explanation of these disturbances in synthesis, especially in the case of overstimulated muscle and rigor. The disturbances after pancreatectomy may however be due to a change in the form in which the glycogen is bound in muscle or an interference with the preliminary storage of the glycogen.

The evidence of synthesis of glycogen by normal muscle during the course of the fluoride action and the absence of such evidence in the case of diabetic muscle are of importance, but further experiments are certainly necessary in order to elucidate the nature of the changes which occur during the progress of the fluoride action.

In conclusion, the muscle of the cat may be regarded as normal if it shows the following properties

(a) In the existing condition

- (1) A lactacidogen value not less than 0.18 p.c. ( $H_3PO_4$ )
- (2) A lactic acid content of 0.08–0.2 p.c.
- (3) Glycogen 0.5 p.c. and over

(b) After hydrolysis

- (1) Lactic acid 0.5–0.8 p.c. (or over)
- (2) Total phosphate 0.48 p.c.

(c) After synthesis of the intrinsic components a lactacidogen value of not less than 75 p.c. of the total phosphate

(d) In the synthesis of the intrinsic and added free phosphate and glycogen

- (1) 1 gm. muscle transforming 11–12 mg.  $H_3PO_4$  (as phosphate) out of 13 mg. from the free to the bound form with
- (2) Practically complete consumption of the glycogen

The factors which indicate disturbances in the metabolism such as are met with in severe pancreatic diabetes are

- (1) A low original lactic acid value (0.03–0.06 p.c.)
- (2) Lactacidogen  $H_3PO_4$  below 0.17 p.c. in the original muscle
- (3) A lactic acid maximum after hydrolysis of less than 0.4 p.c.

(4) Lessened synthesis of both the intrinsic and the added phosphate with the carbohydrate component along with

(5) A tendency to hold up the glycogen during the synthetic processes

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## ON THE REGENERATION OF BLOOD AFTER HÆMORRHAGE

By D T BARRY AND D S TORRENS

WE have performed some experiments on dogs to determine whether or not the regeneration of blood after hæmorrhage is stimulated by the use of fresh liver substance from a separate animal. As this work has to be postponed for some time we have thought it advisable to publish a short account of what has been done up to now, chiefly because of an unusual result remarked in one animal on which liver grafting was performed.

*Method* The liver substance has been employed in various ways. A young healthy dog was taken and bled to death. Boiling water was poured over its thorax and abdomen, so that the epidermis could be removed with an ordinary sterilised knife. This procedure was followed by washing with chinisol lotion which completely sterilises the skin. Hands and instruments being sterilised in the usual way the liver was cut down upon and extracts or grafts were made aseptically from it. The extracts employed were (1) thermostable, made by boiling and filtering, which was injected into a vein, (2) albuminous, made in the cold by grinding with sand in a sterilised mortar and filtering, which was injected into the peritoneal cavity, (3) perfusate, got by perfusing Ringer's solution through the liver vessels, also injected into a vein. Equivalent quantities of Ringer's solution to those of the extracts were used for the control animals in a similar way. A separate control was used for each experimental dog, except in the case of Nos. 22 and 24 for which one control was employed (No. 23). We had used these extracts for some time before trying grafts. For this latter purpose a piece of liver of about 30 grm. was excised with all due precautions, and without weighing, so as to avoid this possible source of infection, it was transferred to the wound in the neck of the experimental dog. On the first occasion the bit of liver was preserved for a few minutes in Ringer's solution (sterilised) before being transferred to the site of implantation.

For the test of regeneration the quantity of blood taken in the first instance was always 3 p.c. of the body weight. More than this amount leaves the animal in very weak condition with slow reactive powers.

The site chosen for bleeding has been varied, the most common being the external jugular vein, but we experienced considerable difficulties in some instances in getting off a sufficient quantity by this vessel in a reasonable time, and in these recourse was made to the carotid or femoral artery. When bleeding is slow a sample of the last blood taken after a loss of 3 p c of the body weight may show considerable differences from one taken at the outset of bleeding. With arterial bleeding, which yields the requisite amount very quickly, the difference between two such samples is negligible. It is important that the rate of bleeding should be uniform in controls and experimental animals, so that no difference of water or lymph absorption from tissues should occur, it is essential for the conditions of analysis of samples and for uniformity of effect on the animals. With regard to the method of analysis simple estimations have been relied on for routine work: the viscosity, specific gravity, corpuscular volume and red cell-count have been determined as a routine procedure, and the proteins and fats of the plasma have been ascertained in some cases. The microscopic appearance of the formed elements has also been examined, and variations in the white cell-count noted. The viscosity was always measured at 20° C and the same viscosimeter used throughout (water time at 20° C 4.8"). We have tried some determinations of volume by the dye method described by Whipple and his co-workers<sup>(1)</sup> as well as others, but we have not had satisfactory results. The technique of the method is difficult, and the great tendency to hæmolysis in the dog is an upsetting factor. The hæmoglobin is very easily dissociable from the globule and colours the plasma. Notwithstanding Lindhard's contention that a small amount of hæmoglobin in the serum does not affect the estimation of volume<sup>(2)</sup> we have not been able to get reliable results because of this factor. While it may sometimes be desirable to know the volume of the circulating blood in this class of work we do not consider it indispensable as a routine measure. It is well on the other hand to ascertain the blood pressure from time to time, and this has been done in these experiments.

Healthy animals have been chosen for the work and great care has been taken in their treatment after operation, special precautions having been adopted for artificial warmth during the first twenty-four hours. After this interval they lived in uniform conditions in the kennels, being fed on biscuits and porridge with occasional scraps of meat and some soup. The kennels were quite dry and warm, and the animals were allowed to go free in the yard from time to time.

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Healthy animals have been chosen for the work and great care has been taken in their treatment after operation, special precautions having been adopted for artificial warmth during the first twenty-four hours. After this interval they lived in uniform conditions in the kennels, being fed on biscuits and porridge with occasional scraps of meat and some soup. The kennels were quite dry and warm, and the animals were allowed to go free in the yard from time to time.

*Results*

*A Injection* Injection of extracts was performed on nine dogs, six intravenously and three intraperitoneally. A control animal in each case was similarly treated by Ringer's solution with or without a little gum or blood to equal the extract employed. In these cases no appreciable differences in composition of blood as between experimental animal and control were noted in the course of recovery during several weeks. One injection only was made in each of these experiments, that is immediately after bleeding. It may be that a stimulus to hæmogenesis was provided by the liver extract, but in such small amount as to produce no appreciable effect. It would be worth while to repeat the injections and to keep up a regular supply during the early period of regeneration after bleeding. We have not been able to adopt this procedure, for which the liver of other animals than dogs might be tried.

*B Grafts* The grafts have been employed in three animals only and were all implanted in the subcutaneous tissue of the neck over the left external jugular vein. In one of these three the wound became distinctly septic, and although complete recovery took place it is looked upon as an experimental failure. It may be remarked that the blood of this dog showed no signs of benefit as compared with that of the control, the serum contained a large amount of fat, which shaken out with ether deposited on evaporation of the ether large masses of cholesterol scales and needles. In the second case there was considerable swelling, non-purulent discharge from and irritation of the wound after grafting, but again complete recovery took place. As in the first dog the blood of this one showed no improvement in specific gravity, corpuscles etc., but the serum was quite clear, the fat was not estimated. In the third animal grafted the wound remained perfectly clean and healed by first intention, although the jaws and lips were swollen for some 48 hours. It seems that the fresh liver tissue, even when quite sterile, is capable of setting up irritation which passes off in time, a mild inflammation without sepsis. This animal was very well on the second day, and the blood condition soon became quite good as compared with that of the control (*v* Tables). The symptoms in general shown by animals after bleeding are pretty uniform, they remain weak and listless for some days, showing little desire for food though thirst may be pronounced. Those which have received injections of gum equivalent to blood loss are more quickly restored than others. The disposition of the animal also shows a change after bleeding, the com-

bative or quarrelsome one becoming subdued and the snappy one quite docile, while there is little tendency to frisk or play, and this condition may last many days. In the third graft dog mentioned above there was a distinct difference in this respect, it soon grew lively and alert, with a disposition to establish an authority over the others when let loose together.

Having noted many disturbances as a result of taking large samples of blood for analysis during the first week or two after bleeding, we were content in this case and that of the control to examine an occasional drop from an ear vein for corpuscle count until the 20th day, large samples were then taken, the results of examination of which are given in Tables I and II. By an oversight the early counts of these two animals were not entered up in the notes. Notwithstanding this we consider the figures given in the tables of sufficient interest for publication.

TABLE I. Control for Nos. 22 and 24.

No	Weight k.	Date	Bleeding c.c.	Specific gravity	Viscosity (20° C.)	Red cells m.	Corpuscles volume p.c.
23	12	28. iv 28	360	1060	3.4	7.4	50
Do	11.9	18 v 28	75	1052	2.75	6.3	35.5

TABLE II. Dog grafted.

No.	Weight k.	Date	Bleeding c.c.	Specific gravity	Viscosity (20° C.)	Red cells m.	Corpuscles volume p.c.
24	20	29 iv 28	600	1061	3.6	8.5	52
Do	19	18 v 26	150	1060	3.4	8.2	48

Table I represents a fairly typical control in which recovery is slow, and 20 days after the bleeding the blood is still thin. Discrepancies in the relation of corpuscle bulk to numbers are frequent, and the variations suggest that the volume of corpuscles undergoes rather quick changes during the course of regeneration. The difference of one day in the dates of this control dog and the experimental one (24) is explained by the fact that No. 22, grafted on the same day that the control was bled, showed a poor condition on the morrow of the operation, and so a second dog was bled and grafted on this latter date.

*Remarks.* The work of Robschert-Robbins and Whipple(3) in feeding bled dogs on liver substance suggests that this contains a principle which makes for regeneration of hæmoglobin and corpuscles. The present method of investigation seems to us, if not better adapted than feeding to elicit the truth or otherwise of this proposition, at least to be a very useful accessory line of investigation. Although we have only one successful experiment to present in favour of the suggestion, the



result in this one instance is marked. About an ounce of fresh liver substance was implanted under the skin of a bled animal and in the course of three weeks the hæmoglobin and corpuscles were restored, as were the viscosity and specific gravity of the blood, in much higher proportion than in the case of a control treated in every respect similarly except for the use of liver substance. The graft at the end of three weeks was examined post-mortem and showed the following condition. The consistence of the residue was of a semi-fluid nature, and this remnant amounted to about 1 grm. It was shut off by a sort of soft connective tissue from the surroundings. Microscopically this substance showed no formed elements, consisting chiefly of débris and granular fluid with a few flakes of vaguely fibrous material.

Our observations are admittedly incomplete, and we recognise that much more work is needed to carry us clear of the realm of hypothesis. But the analysis of the samples of blood was carried out with much care, the specific gravities were measured directly with a urinometer, the corpuscle volume was uniformly determined in the oxalated blood, and counts were carefully made with a Burgher slide after macro dilution (1 c c in 99 c c Ringer's solution). Accordingly no serious error can have occurred in our examination of the samples.

We do not know what value to attach to the result in the second dog grafted (No. 22), the degree of sepsis, if it may properly be so called, was much less than in the first, but the wound did not heal at all so readily as in the third, while the liver tissue, apparently because the reaction which occurred in the surrounding tissues did not tend to preserve it, seems to have melted away very quickly. Tentatively we have considered it as an instance of sepsis and so refer to it in our conclusions.

#### SUMMARY AND CONCLUSIONS

Nine dogs were bled to the extent of 3 p c of the body weight and given one injection of liver extract intravenously or intraperitoneally. No appreciable differences were noted in their blood from that of the controls in the course of recovery.

Three dogs were bled to the same extent as the above and treated by grafting liver substance in the subcutaneous tissue of the neck. Two of these developed sepsis and showed no improved regeneration of hæmoglobin as compared with controls. In the third animal the wound healed by first intention, and it showed better regeneration of hæmoglobin than the control. In recording this experiment we merely desire to suggest

that it lends a little support to the theory that the liver possesses the property of stimulating hæmogenesis. Possibly similar properties exist in other tissues.

NOTE. Part of the expenses of this research were defrayed by a grant to one of us from the Medical Research Council.

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# ON THE INNERVATION AND SECRETORY PATH OF THE THYROID GLAND

By C S HICKS (*Best Memorial Fellow*)

(*From the Biochemistry Laboratories, Cambridge, and Physiology  
Department, University of Adelaide*)

IN an attempt to develop a precipitin test for the presence of thyroid secretion in the blood of "hyperthyroid" patients, it was found that thyreoglobulin prepared by Oswald's method, acted as a powerful antigen when injected into the blood of the rabbit. The present work had already passed its preliminary stages in conjunction with chemical work in the same field(2) prior to knowledge of the first paper on thyreoglobulin published by Hektoen and Schulhof(1), and since the appearance of an interim report(3) has been further extended. The present paper includes all work up to the present, and serves both as an extension and a corroboration of the work of Hektoen and Schulhof who, however, worked with markedly goitrous animals(2).

On the assumption that thyroxin would doubtless be secreted into the blood or lymph, combined with the globulin originally shown by Oswald to contain the total activity of the gland, it was proposed to apply the precipitin test for thyreoglobulin to the examination of blood and lymph from the thyroid in the intact animal.

Most of the work on the innervation and control of secretory activity of the thyroid gland has given rise to indirect, or contradictory results capable of interpretation according to prejudice. The anatomy of the nerve supply is clear—namely, that sympathetic nerves to both vessels and gland tissue travel by the superior laryngeal nerve to be distributed along the superior thyroid arteries(4), but removal of the nerve supply seems to leave the gland function unimpaired. Using tests for iodine in the thyroid venous blood van Dyke(5) obtained negative results. Cannon(6) showed that electrical changes in the gland could be produced by stimulation of the thyroid nerves, but his work on the anastomosis of phrenic and sympathetic producing changes in gland structure, has not been corroborated(7). His further work on stimulation of the

nerve supply to the gland and its effect on the denervated heart<sup>(8)</sup> raises the difficulty of explaining the observed rapid increase in cardiac rate, all the evidence so far available pointing to delayed action on the part of thyroid secretory products. The possible route of the secretion has been decided only on histological and cytological grounds<sup>(9)</sup>

Preliminary work on the lymph drainage of the thyroid gland in the dog was begun under the guidance of Dr F C Mann at the Mayo Institute of Experimental Medicine, Rochester, Minn., in the summer of 1924

Thyroids from recently killed animals were rapidly washed with water (while being torn into small pieces) until almost colourless. The fragments, free from all fat and connective tissue, were then ground lightly with incinerated beach sand in normal saline containing thymol dissolved in toluol. The mixture was allowed to stand at 0° C for three days until the thyreoglobulin had dissolved in the saline, and was then thoroughly filtered (see Hektoen and Schulhof). The filtrate was then treated with an equal volume of saturated ammonium sulphate and the precipitate well washed with 50 p c ammonium sulphate in normal saline, and re-precipitated, the operation being twice repeated. The precipitate was then dialysed against distilled water in a collodion sac until free from ammonium salts as determined by nesslerising the dialysate. The solution was then evaporated rapidly *in vacuo* to dryness and solutions of thyreoglobulin made up by dissolving the dried globulin in normal saline to 1 p c concentration. 10 c c injections of this solution were made into the ear vein of the rabbit on four successive days, repeating the operation after an interval of ten days. During the week following the last injection the titre of the serum reached its maximum of 1:80,000. Serum precipitins, arising from serum contamination in the antigen, were removed by adding to the antiserum equal parts of 1:250 dog serum in saline, standing for four hours and centrifuging off the precipitate so formed. Such antiserum gave no precipitate when treated further with dog serum. The tests were made by the layer method, the antiserum being added below the surface of the solution to be tested, the results being read by suitable light after one hour at room temperature. Progressively diluted antigen was used in order to avoid failure of reaction due to high concentration.

*Collection of Lymph* Warmed ether was carefully administered by means of a tracheal cannula and the main lymphatic vessels of the neck were displayed by blunt dissection and ligated as low in the neck as possible. The lymph from the gland drains by several channels to a

node near the angle of the jaw, and above this the trunks draining the head were ligated, the filling of the lymphatics after the lower ligature increasing the visibility of the otherwise extremely delicate vessels. It is clear that the ligated vessels will contain thyroid lymph plus some neck lymph, and a cannula inserted in this vessel supplied the material to be tested. See Carlson and Woelfel for details of technique<sup>(10)</sup>

Lymph was collected for a period of three hours, during which time the anaesthesia was uniform and lightly maintained. From the two glands a total amount of 0.4 c.c. of lymph was obtained in the above period, representing an average flow of 1.6 c.c. per gland per 24 hours.

*Test* The lymph was mixed with an equal volume of 1:250 dog serum in 0.9 p.c. sodium chloride solution and after standing for four hours at room temperature, the precipitate was removed by centrifuging at 3000 r.p.m. The dilutions of lymph in saline were arranged in small sedimentation tubes, and to 0.2 c.c. in each tube was added carefully at the bottom of the tube 0.2 c.c. antiserum. After standing for an hour at the room temperature these tubes were examined for precipitate at the plane of contact of the solution. Table I shows the result obtained with animal A compared with pure antigen solution.

TABLE I.

	Thyreoglobulin solution	Thyroid lymph	Blood serum
Anti dog thyreoglobulin serum alone	1:80,000	1:260	1:120
Same after adding equal vol. 1:250 dog serum and centrifuging	1:60,000	1:20	No action

TABLE II.

Dog	Thyroid lymph	Thyroid blood	Arterial blood
A	1:20	1:15	No action
B	1:12	1:15	"
C	1:18	1:18	"
D	1:24	1:20	"
E	1:30	1:18	"

NOTE: Serum precipitans removed before testing

It is clear that thyroid lymph and venous blood contains a substance which reacts with an antiserum against dog thyreoglobulin, while this substance is absent from arterial blood, or too dilute to react. In the case of animals D and E 60 minims of the B.P. tincture of iodine in capsules had been administered before the operation. This was done to further test the accuracy of an observation originally made by Mann, and further confirmed by Mann and Hicks during the preliminary work to this study, that iodine administration caused an increase in lymph

flow from the thyroid in the dog In the present case the same observation was made—the lymphatics stood out more plainly after ligaturing, and felt tense, and the flow was more plentiful than in the other cases, being 0.5 and 0.6 c.c. per gland respectively It will be noted that there is an apparent slight increase in the strength of the reaction with antiserum, but the extent of the experiment warrants no further conclusion. Further work is necessary to establish this observation as being constant, and is in progress at present

Stimulation of the cervical sympathetic<sup>(11)</sup> was carried out and the lymph from the stimulated side compared with that from the other side in animals B, C, D and E No change in titre could be established either between the lymph of the same side before and after stimulation, or between the stimulated and non-stimulated sides

It would appear from these results that thyreoglobulin finds its way more by the blood stream than by the lymphatics, the rate of flow in the latter being very slow compared with that of the blood in the veins, and the concentration being of the same order in both No conclusion can be drawn as to the effect of possible secretory fibres in the sympathetic nerve supply to the thyroid, although it might be urged that the effect might be slower than the nature of the experiment could detect These results confirm those of Carlson, Hektoen and Schulhof<sup>(12)</sup>

#### SUMMARY AND CONCLUSIONS

1 Secretion of thyreoglobulin into blood and lymph has been studied in dogs with normal thyroids

2 Thyreoglobulin as an index of thyroid secretion passes from the thyroid gland to both lymphatics and veins, chiefly by the latter route

3 Electrical stimulation of the sympathetic nerve supply to the thyroid of the dog produces no change in thyroid secretion rate as measured by the output of thyreoglobulin determined by the quantitative precipitin reaction of the latter

4 Administration of iodine appears to increase the flow of lymph from the apical lymphatics of the thyroid gland as well as to increase the content of thyreoglobulin therein This latter is a conclusion based on experience with four animals and is tentative pending further work on the subject

My thanks are due to Dr F. C. Mann of the Mayo Foundation, Rochester, Minn., and also to Dr G. Scott Williamson of the Dunn Laboratory, St Bartholomew's Hospital, who secured for me the dog

thyroids used in the preparation of the thyroglobulin for these experiments

The work has been carried out during the tenure of a Beit Memorial Fellowship for Medical Research, and was done under the auspices of the Medical Research Council.

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# THE ANAEROBIC DELAYED HEAT PRODUCTION IN MUSCLE BY K. FURUSAWA AND W. HARTREE<sup>1</sup>

*(From the Physiological Laboratory, Cambridge)*

DURING the past nine months we have carried out over 100 experiments in order to verify the existence and to determine the amount of the anaerobic delayed heat occurring in isolated frog's muscle after stimulation. Hartree and Hill(1) concluded that this is about 0.25 of the total initial heat, but in view of the importance of this quantity it seemed desirable to remove any possible doubt concerning it. The method employed was the same as that described in the above paper, but in all the later experiments a special thermopile with two faces was used which will be described later.

The Ringer's solution which filled the chamber initially had been carefully boiled to exclude oxygen, and the nitrogen employed was freed from oxygen as completely as possible. The quality of the nitrogen was frequently tested. On a few occasions it was found to contain about 0.1 p.c. of oxygen, but usually it contained about 0.05 p.c. or less.

In several cases we found that when such purified nitrogen was passed through the muscle chamber for an hour or so after the Ringer's solution had been blown out, the muscle became slowly inexcitable, as Bayer(2) and Fröhlich(3) have shown in the case of nerve. In such cases a complete recovery could be brought about by soaking the muscle for a short time in aerated Ringer's solution. A certain minimum, therefore, of oxygen (less than 0.1 p.c.) is necessary to keep the muscle excitable. This state of inexcitability evidently started on the side of the muscle exposed to the nitrogen, producing peculiar results which should be mentioned. When the outside of the muscle is inactive, the temperature of that part will not rise immediately on stimulation, so part of the heat produced in the inside (and near the thermopile) must flow to the outside soon after the stimulus. The thermopile, therefore, will be affected in exactly the same way as if there were an absorption of heat by the muscle following shortly after the initial rise of temperature. In other words, there will be an apparent negative heat production soon after the stimulus. This was verified long ago by

<sup>1</sup> Working for the Medical Research Council.



scorching the surface of the muscle with a hot wire, records taken after this showed an apparent large negative heat soon after the contraction. Of course if this negative heat be large, or if it occur early, it may affect the maximum deflection itself (especially when using a slowly acting thermopile), and since the delayed heat is determined by the difference of the "live" curve and the "control" curve when these are reduced to the same maximum—which would be correct if the muscle were uniformly heated when it contracted—it can be seen that if the live curve has a false maximum the effect on the above difference may be relatively large. Apart from this possible error due to a false maximum, the state of inexcitability spreading inwards from the exposed surface is sometimes well shown by the increasing "negative heat" after successive stimuli.

On the other hand, in many experiments we observed that there was a large early positive difference between the live curve and the control curve, as observed by Hartree and Hill (1), p 133. This was specially evident when using longer stimuli (0.3 sec or more) as was done in that case—in fact we have several experiments in which this happened, and yet when a short stimulus (0.05 sec or less) was given, the early apparent *negative* heat was very evident. These irregularities can possibly all be explained by supposing that the heat is not produced uniformly across the section of the muscle on stimulation, due possibly either to the over-stimulation of some of the fibres or to the inactivity of others. In general it is probable that the inside of the muscle will remain in better condition than the surface, which may have been damaged during preparation or affected by contact with the thermopile.

During the course of our experiments Prof Otto Meyerhof suggested that we should make some observations on the effect of very strong stimuli on the delayed anaerobic heat. A preliminary statement of our results was published in Pflüger's *Archiv* (4). There we showed that after a strong faradic stimulus (containing five to eight times as much energy as a just maximal stimulus) there occurs an anaerobic delayed heat amounting to 20–60 p.c. of the initial heat.

Subsequently we have made several experiments with an extra strong stimulus of short duration (0.05 sec). The energy of this stimulus was several times greater than that in the initial response of the muscle. In this case, following the initial heat production there is a relatively enormous outburst of delayed heat, the total extra heat produced during a period of 2 or 3 minutes after the stimulus being well in excess of the initial heat itself. This delayed heat, which cannot be regarded as

associated with a normal muscular contraction, but rather as due to injury produced by the excessive stimulation, is obviously to be associated with the delayed lactic acid formation found by Embden(5) and discussed by Meyerhof and Lohmann(6)

Such "electrocution" of the muscle is of little interest in connection with normal muscular contraction, but it is obvious that measures must be taken to avoid its effect when employing direct stimulation of muscles, and the question necessarily arises whether those fibres of a muscle which are immediately in contact with electrodes do not themselves receive excessive stimuli even when, for the muscle as a whole, the stimulus is only just maximal.

Returning now to the case of ordinary stimuli, when there is apparent negative heat soon after the stimulus its amount invariably increases with successive stimuli of the same duration, and in general the size of the curve of delayed heat rate falls slightly for successive stimuli, the first curve being often abnormally high, due possibly to traces of oxygen.

Since in these anaerobic experiments we have observed many cases of apparent early negative heat and also of apparent early positive heat occurring after contraction is over, it was inevitable in a long series of experiments that there would be several cases in which the live curve and the control curve were practically identical for some time (half a minute or more) from the start. If any other state of affairs is taken as being due to experimental error, these cases are entitled to the greatest weight, although even then it might have happened only because the positive and the negative errors were initially equal. In many cases there was only a small early negative heat or a small early positive heat above that due to a smooth curve starting from time zero. In such cases an attempt was made to correct the result. Early positive heat was estimated by analysis of the early part of the curve, and this was deducted from the observed difference in the areas of live and control curves. Similarly, if there was apparent early negative heat, this was estimated by analysis and added to the observed difference in the areas of live and control curves. Such corrections, however, were made only when they were not more than 2 or 3 p c, in exceptional cases they might be as much as 8 p c. (For numerical results see later.)

We are admittedly attempting to find the minimum value of the anaerobic delayed heat. For this reason about a quarter of the experiments which gave a delayed heat of between 20 and 30 p c of the initial heat (as found by Hartree and Hill) have been discarded,

although the nitrogen used in these experiments was of good quality and there was no special reason to suspect the results. In a further quarter of the experiments the early negative or extra-positive heat was too large to be taken into account with any accuracy. Even so, however, we have 40 reliable experiments on which to base our results.

Owing to the occurrence, especially in the purest nitrogen, of large apparent negative heat, which, as explained above, was probably due to the inexcitability of the outside of the muscle on the ordinary thermopile, Prof A V Hill suggested a new type of thermopile, which was made by Mr Downing of University College, London, and consists of two parallel thermopile plates joined in series, with the muscle lying between them so that only its edges are exposed. Since a single sartorius muscle was rather small and thin to warm both faces of this thermopile, a semi-membranosus muscle was used in all the experiments with it. The thinness of the insulation and the good contact of the muscle with this type of thermopile caused the heat to be transferred very quickly to the hot junctions, with the result that the maximum galvanometer deflection was reached in about 3 seconds, even with a large muscle weighing 0.3 gram or more. Moreover, loss of heat from hot to cold junctions was rapid. These facts would have rendered technically very difficult an actual *analysis* of the heat rate throughout the record, and none was actually made, only the total area of the deflection-time curve being determined in order to ascertain the total delayed heat.

This thermopile did not entirely eliminate the negative heat—in fact in all the cases in which a short stimulus (0.03–0.05 sec) was used it showed 2–4 p.c. early negative heat, with a longer stimulus (0.3 sec) the same muscle never showed any early negative heat, though usually there was some early extra positive heat in these cases. In all the experiments made with this thermopile the nitrogen had about 0.3 p.c. of oxygen present in it, and in only one or two cases was there obviously increasing negative heat for successive stimuli. The very small surface of the muscle exposed cannot have allowed any perceptible amount of oxygen to pass in, probably only sufficient to maintain the excitability.

The numerical results from 40 fairly reliable experiments, all at 18° C in nitrogen, including those using both types of thermopile and employing various times of stimulus from 0.03 to 0.3 sec, were as follows. In 12 experiments with no correction for early irregularities the mean total delayed heat was 12 p.c. (one result over 13 and one under 10), in 12 experiments with not more than 3 p.c. of early positive heat the mean total delayed heat after deduction of this early positive heat was

11½ p c (two results over 14 and three under 10), in 16 experiments with not more than 3 p c of early apparent negative heat the mean total delayed heat after addition on this early negative heat was 12½ p c (three results over 14 and three under 10)

The minimum value, therefore, of the anaerobic delayed heat now appears to be reduced to 12 p c, with the reservation that this result is obtained by excluding a considerable number of experiments, apparently reliable, which gave a much greater result, and a few which gave a slightly less result, taking all the experiments into account the mean is roughly 15 p c, but, as Prof Hill suggests, it is quite possible that the anaerobic delayed heat is not a constant fraction of the initial heat, but depends upon conditions. At first sight it might be supposed that this number, which was about 50 p c according to Hartree and Hill in 1922(7) without using specially prepared nitrogen, and about 25 p c according to their re-determinations in 1923(1), should in reality be zero. Progressive improvements in the technique seemed always to reduce the figure for the anaerobic delayed heat. We cannot agree, however, with such a conclusion. Our observations seem definitely to prove the existence of some such delayed heat, since in all cases, even in the purest nitrogen, *the galvanometer deflection after a stimulus persists much longer than in the case of the control curve*. No initial irregularities of heat production between different points of the muscle could possibly have any effect on the deflection after the time taken by the control curve to return to zero. Deflection after that time must mean heat production going on after the contraction is over, and the amount of that deflection is much larger than can be accounted for by any possible experimental error. At 18° C the deflection is observable in the curves of the live muscle up to 11 or 12 minutes, whereas in the control curve it has reached zero in about half that time. The maximum deflection was always about 500 mm., readings were made to the nearest half-millimetre and are certainly correct to the nearest 1 mm. The greatest difference between the live and the control deflections, in the cases giving the smaller total of delayed heat (10–15 p c), was usually from 6 to 8 mm. and 2 to 4 mm., deflection persists in the live curve at a time when the control curve has returned completely to zero.

Another reason for regarding this anaerobic delayed heat as due to causes within the muscle, is the fact that at 0° C its rate is considerably diminished (see Appendix), which would not be the case were it due to purely physical causes of any kind.

As to the origin of this anaerobic delayed heat we have no very

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As to the origin of this anaerobic delayed heat we have no very

definite suggestions to offer. It is conceivable that an ordinary maximal stimulus may be supermaximal for the fibres in immediate contact with the electrodes and that these fibres then exhibit the delayed heat production shown above to result from excessive stimulation. It would seem unlikely, however, that a sufficient number of fibres should be so over-stimulated as to produce the amount of extra heat actually found. It would appear more probable that there is a genuine delayed anaerobic heat of normal physiological origin, for which some explanation must be found in the mechanism of the muscle. It is now commonly believed that a hexose diphosphoric ester is the immediate precursor of the lactic acid liberated during stimulation, and it would seem possible that the re-formation of this compound, at the expense of phosphate and glycogen, occurring only slowly in the almost complete absence of oxygen, may be the origin of this delayed anaerobic heat.

If the number 12 p.c. for the delayed anaerobic heat be now accepted as of genuine physiological origin, and the revised value of 390 calories taken for the heat per gram of lactic acid liberated during contraction (Meyerhof(8)), then the "balance sheet" of Hartree and Hill (1), p. 135) must be revised as follows

	Relative	Absolute calories
Total anaerobic	1.12	390
Initial	1.00	348
Delayed anaerobic	0.12	42
Delayed oxidative	1.50	522
Total oxidative	2.50	870

Employing Slater's latest value(9) for the heat of combustion of 1 gram of dissolved glycogen, namely 3836 calories, this result implies that only  $\frac{870}{3836} = \frac{1}{4.4}$  of each gram of lactic acid liberated during contraction is actually oxidised, or in other words, 4.4 molecules of lactic acid are removed in recovery for each one oxidised.

If the number now found, namely 12 p.c., for the delayed anaerobic heat be still attributed to error, a conclusion which we regard as improbable, then the balance sheet is as follows

	Relative	Absolute calories
Total initial	1.00	390
Delayed oxidative	1.50	585
Total oxidative	2.50	975

This implies that  $\frac{3836}{975} = 3.94$  or approximately 4 molecules of lactic acid are removed in recovery for each one oxidised. Either of these

values, 4.4 or 4.0, is in close agreement with Meyerhof's results in the extensive series of researches he has carried out on the subject

## APPENDIX

Several analyses were made, using the slow acting thermopile, of the rate of delayed heat production at 18° C. The results usually showed a maximum rate of about 0.0006 initial heat per second, as previously found (1, p. 133), but this maximum occurred earlier than before (at about 80 or 90 seconds after the stimulus) and the fall after the maximum was more rapid, making the area of the curve only about half that previously obtained.

In addition a few experiments were carried out at 0° C., in this case the maximum rate of delayed heat production was rather less than half that at 18° C. and the maximum probably did not occur before 3 minutes after the stimulus. In such cases it is impossible to estimate the total delayed heat but the form of the curve for the first 5 or 10 minutes showed that the total delayed heat was not going to be reduced much, if at all, by lowering the temperature.

A further set of experiments was made when the muscle was kept in hydrogen. These are not so reliable as those in nitrogen, this is partly due to the high conducting power of the hydrogen making the deflection curves have an earlier maximum and a much smaller area (for the same maximum), and partly because there was a greater proportion of these experiments in which there was apparently a large early negative heat and this may have been due to some specific effect of the hydrogen, the results of a few good experiments seemed to show about the same total delayed heat as in nitrogen, but the maximum rate was usually smaller, and at a longer time after the stimulus than when nitrogen was used.

Lastly, we tried eliminating the oxygen by keeping the muscle in a partial vacuum exhausting the muscle chamber until the Ringer's solution boiled at room temperature. In this case, however, we obtained no reliable results, probably because relatively large temperature changes were brought about by rapid evaporation from the surface of the muscle after stimulation and, further, the difficulties referred to in the case of the hydrogen experiments were exaggerated.

## SUMMARY

1 The delayed anaerobic heat production in muscle has been re-investigated. A new double-faced thermopile designed to lie in contact with the muscle on both sides was used in the later experiments.

2 The minimum value of the total delayed anaerobic heat is shown to be about 12 p.c. of the initial heat. It is probable that this heat has a physiological origin in the normal mechanism of the muscle and is not to be attributed to any constant source of error.

3 Accepting this value it can be calculated that 4.4 molecules of lactic acid are removed in recovery for each one oxidised.

4 An excessive stimulus, directly applied, may cause a large delayed anaerobic heat attributable rather to injury by the stimulus than to normal physiological processes.

5 The delayed lactic acid production found by Embden in muscles directly stimulated is probably to be associated with the delayed heat found under such conditions of excessive stimulation.



We are much indebted to Prof A V Hill for his suggestions and help during the experiments Our thanks also are due to Mr A C Downing for his construction of the new double thermopile One of us (K F) is indebted to the late Prof Langley and to Prof Barcroft for their kind permission to work in the Cambridge Laboratory

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# PROLONGED ALTERATIONS OF OXYGEN PRESSURE IN THE INSPIRED AIR WITH SPECIAL REFERENCE TO TISSUE OXYGEN TENSION, TISSUE CARBON DIOXIDE TENSION AND HÆMOGLOBIN

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ALTHOUGH a great deal of research has been published regarding the effects of acclimatisation to low  $O_2$ -pressure in the inspired air upon respiration and upon the respiratory function of the blood, there is no definite information regarding its effects upon the  $CO_2$ - and  $O_2$ -tension in the tissues. The experiments described in the present paper were undertaken to study this problem near sea-level and also the effects of acclimatisation to high  $O_2$ -pressures, employing the method of injection of gas for estimations of the tissue  $CO_2$ - and  $O_2$ -tensions.

*Technique* The apparatus (Fig 1) employed was similar to that used by Edie(1). The chamber was large enough to allow freedom of movement for the animal (rabbit), the dimensions of the chamber being height 61.3 cm, length 60.5 cm, width 45.6 cm, and capacity 169.2 litres. The  $O_2$ -pressure in the air in this chamber could be kept at any desired level, the  $CO_2$  produced by the animal being absorbed by soda-lime, fresh  $O_2$  bubbled into the chamber through a water valve from a spirometer containing nearly pure  $O_2$  (98 p.c.) under a small positive pressure. There was about 2-3 p.c. of  $N_2$  in the spirometer and this would have gradually lowered the  $O_2$ -pressure in the chamber, if the capacity of the chamber had not been increased, this was effected by means of a reservoir (16 litres) of water connected with the chamber by a rubber tube, the necessary amount of water being run out an extra amount of  $O_2$  was drawn into the chamber from the spirometer to counteract the increase of  $N_2$ . In his paper Edie gives the simple calculation for the amount of water required to be run out.

By means of  $3\frac{1}{2}$  to 4 kilos of soda-lime spread out in a dozen trays at the top of the chamber, the  $CO_2$  in the air in the chamber was kept below 0.15 p.c. for more than 5 days when a rabbit about 3 kilos was living inside. Food and water for this period were included and the bedding did not require to be changed sooner. The food consisted of

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forcing gently through the chamber the necessary quantity of  $N_2$  or  $O_2$  from a large bag, the quantity required being determined in preliminary experiments

The  $CO_2$ - and  $O_2$ -tensions in the tissues were estimated from the  $CO_2$ - and  $O_2$ -tensions in gas (air or  $N_2$ ) injected—in quantities up to 500 c c and more—under the skin and into the abdominal cavity (2). Samples could be withdrawn from the animal within 2 minutes of opening the chamber, no anaesthetics being employed. It was observed that, in the experiments under increased  $O_2$ -pressure in the inspired air, the injected gas was absorbed more rapidly, particularly from the abdominal cavity, than when breathing ordinary air, the abdominal cavity had therefore to be injected with larger quantities and more frequently that is every few days instead of every two weeks, this procedure did not affect the results for tensions either under the skin or in the abdominal cavity if the animal was accustomed to frequent injections and provided samples were not withdrawn before about 40 hours. The  $O_2$ -consumption was estimated from the  $O_2$  lost from the graduated spirometer and from the  $O_2$ -content of the chamber at the beginning and at the end of each 24 hours, due allowance being made for temperature and pressure. The amount of  $O_2$  consumed by the animal for at least 21 out of each 24 hours was obtained and in the results it has been expressed in c c per minute. Control experiments indicated that there was no leakage from the chamber or spirometer and that there was no significant consumption of  $O_2$  by the food, etc in the chamber. Haemoglobin was estimated with Haldane's haemoglobinometer and the blood cell counts with the Thoma-Hawksley haemocytometer. The barometric pressure was almost constant at 745–760 mm Hg.

Three experiments were performed on separate animals with low  $O_2$ -pressure in the air and two with high  $O_2$ -pressure, employing the same animals as for lowered pressure. In the case of one animal the experiment with high  $O_2$  was carried out first and in the case of the other the low  $O_2$ -experiment was first completed, where comparable the results confirmed one another.

*Limits of alterations in  $O_2$ -pressure in the inspired air.* With regard to lowered  $O_2$ -pressure Edie (1) stated that a rabbit could survive for at least 48 hours whilst breathing  $O_2$  at about 42 mm Hg, i.e. 6 p.c., this he considered was the lowest limit for safety during such periods of time. In my first experiment a rabbit was exposed suddenly to  $O_2$  pressure at about 66 mm Hg (9.4 p.c.). It exhibited marked hyperpnoea, loss of appetite and weight with a gradual fall of  $O_2$ -consumption

a liberal supply of oats and hay, with one small cabbage per week, the bedding, placed on a removable tray (not shown in diagram), was

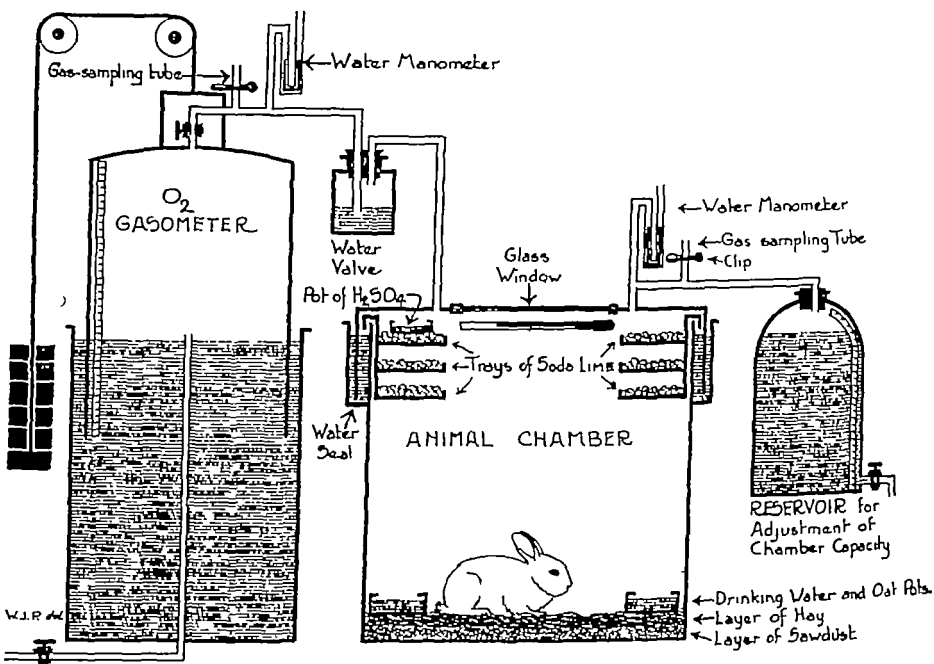
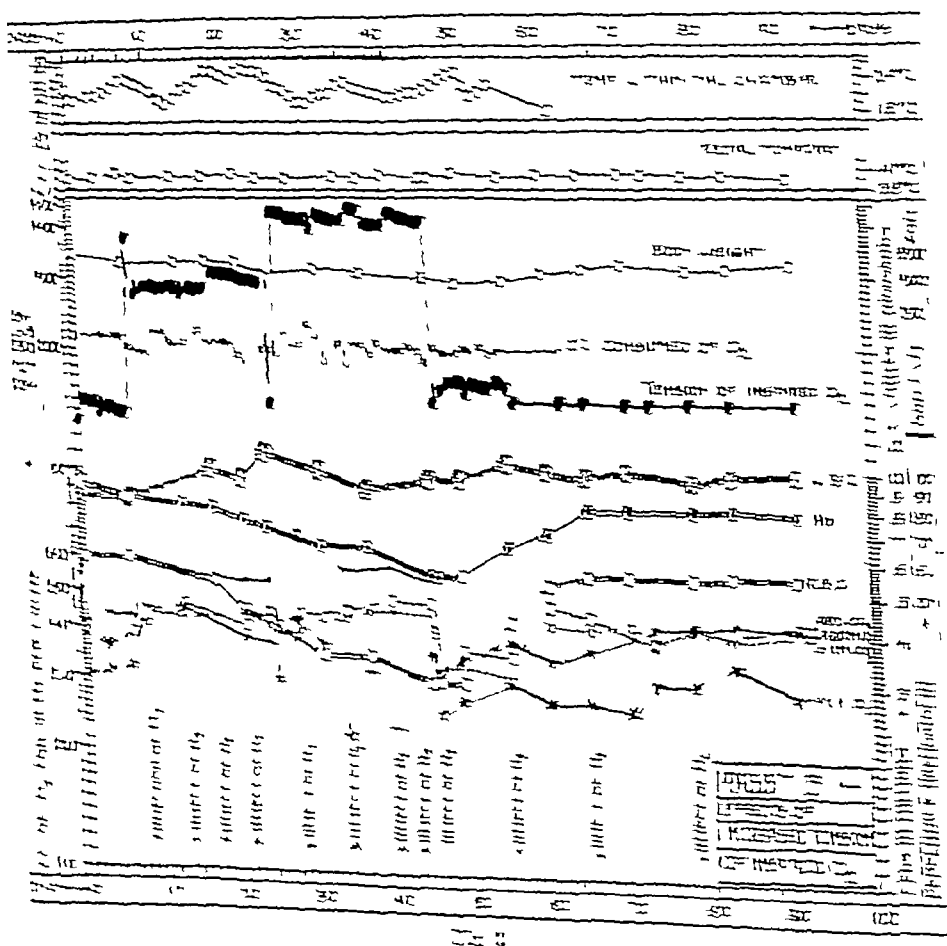


Fig 1

composed of about 3 cm of sawdust covered with about 25 cm of loosened hay. With this bedding no trouble was experienced in keeping the chamber clean for 5 days, although the urine was simply absorbed by the sawdust, there was little evidence of ammoniacal fermentation, only a trace—too small to be estimated—of ammonia was detected in the air of the chamber by qualitative test and small basins of dilute  $H_2SO_4$  were placed in the chamber to absorb this. When the chamber was opened at the end of 5 days there was only the odour of hay noticeable. It was necessary to open the chamber about every 5 days to renew the soda-lime, food and bedding, but by having two sets of trays this could be done in a couple of minutes. When an experiment was being conducted samples of blood and of injected gas had to be taken from the animal, even with this extra work it was possible to put the animal back into the chamber again in about 10 minutes, so that it was only breathing ordinary air for this short time.

The desired  $O_2$ -pressure in the air in the chamber was obtained by



On the whole it remained fairly comfortable for the first 7 days, on the seventh day its  $O_2$ -consumption had fallen by about 25 p c. It developed tetany on the eighth day and although the  $O_2$ -pressure in the air was raised to 81 mm Hg (11.6 p c) it died on the ninth day. Post-mortem examination—10 hours after death—revealed a certain amount of œdema in scattered areas of the lung and a few small areas of necrosis in the liver. The animal had lost 13 p c of its weight in 9 days. From this experiment it would appear that sudden exposure to  $O_2$ -pressure at about 66 mm Hg produced a slow and insidious change and that Edie's figure of 42 is probably too low a limit for prolonged exposures. In another experiment, illustrated in Fig 2, the  $O_2$ -pressure in the air was

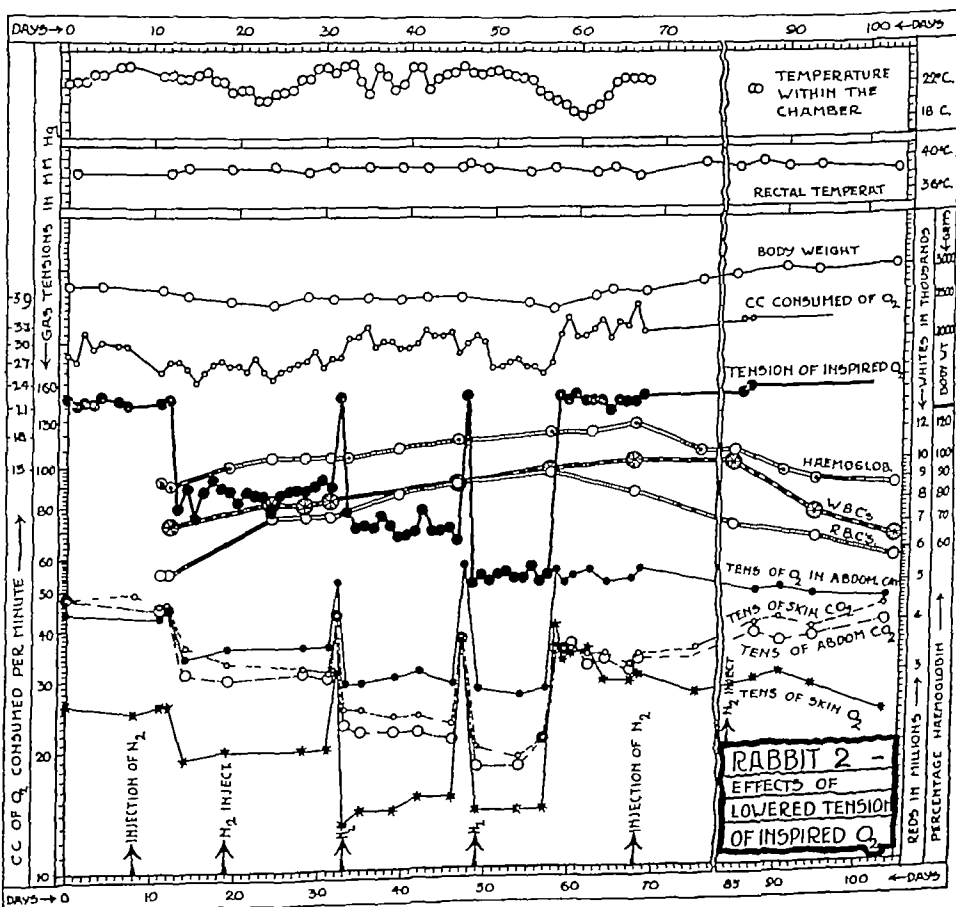


Fig 2.

Prolonged exposure of rabbits to high  $O_2$ -pressure in the inspired air was also without effect upon the metabolism (Fig 3 and Table II). This is in agreement with results obtained for a few hours' exposure.<sup>(5)</sup> The figures in the table are not for basal metabolism, but cover practically the entire period of the experiment.

TABLE II. Rabbit 3 (3.4 kilos), average figures.

$O_2$ -pressure in inspired air mm. Hg	$O_2$ -consumption p.c. per mm.	Duration days	Temp. chamber °C.
141	31.7	7	22.3
291	30.4	18	23.5
416	30.1	20	22.2
160	32.5	9	23.2

*Weight.* There was a gradual loss of weight during exposure to lowered  $O_2$ -pressure in the air with a tendency to return towards the original figure. The maximum loss was about 12 p.c. during exposure to  $O_2$ -pressure at about 53 mm. Hg (Fig 2). loss of weight was made good after the return to normal air but later the animal lost some weight again. loss of weight in man is a common effect of exposure to high altitude. Similarly exposure to high  $O_2$ -pressure in the air caused a maximum loss of about 12 p.c. in weight (see Fig 3) after the return to normal  $O_2$ -pressure the animal's weight gradually returned to normal.

*Hb and red corpuscles.* Prolonged exposures to altered  $O_2$ -pressures in the air had profound effects upon the blood. The main results indicated that the Hb p.c. and the red cell count increased as the pressure in the air diminished, and vice versa, and that the functions of the Hb-controlling organs were regulated by the  $O_2$ -content of the blood. The important facts from the experiments illustrated in Figs 2 and 3 are grouped together in Table III and Fig 4.

TABLE III.

$O_2$ -pressure in inspired air mm. Hg	Red cell millions per cmm.	Hb p.c.	Cell count	Rabbit
53	4.5	120	0.77	2
71	4.0	115	0.78	
85	7.5	105	0.85	
144	5.5	90	1.00	
220	3.0	60	1.22	3
290	2.2	100	0.62	
141	5.0	85	1.00	
291	2.2	72	1.21	
41	3.0	55	1.22	

The experiment with high  $O_2$ -pressure using Rabbit 2 was commenced 108 days after the end of the experiment with low  $O_2$  the



the  $O_2$ -pressure is about 53 mm Hg. According to Haldane's figures for man(3) the alveolar  $O_2$ -tension would be about 35 mm Hg, whilst breathing air with  $O_2$  at 53 mm Hg.

With regard to the opposite condition, that is an increase of  $O_2$ -pressure in the inspired air, Lorraine Smith's experiments(4) indicated that prolonged breathing of  $O_2$  at 490 mm Hg (70 p c) or above caused pneumonia, in my experiments lower pressures than these were therefore employed. The results for one of the two experiments performed are given in Fig 3, the animal being exposed to  $O_2$  at about 291 mm Hg (41 p c) for 18 days and then, after 1 day's interval at normal pressure, to 416 mm Hg (60 p c) for a further 20 days without ill-effects, 291 and 416 mm Hg pressures of  $O_2$  represent about 100 p c and 200 p c increases above normal for inspired air. According to Haldane(3) the alveolar  $O_2$ -tensions would have been about 250 and 370 mm Hg respectively, in place of the normal figure 100.

*$O_2$ -consumption* In previous experiments with unacclimatised immobilised animals a sudden decrease of  $O_2$ -pressure in the air to below 84 mm Hg (12 p c) for a few hours definitely lowered  $O_2$ -consumption(5). This was apparently a temporary effect, since in the prolonged exposure to gradually lowered  $O_2$ -pressure no definite effect upon  $O_2$ -intake was observed (Table I and Fig 2), the figures for some of the periods of exposure to low  $O_2$ -pressure being somewhat above and for others somewhat below the normal  $O_2$ -consumption, the animal—ordinary quiet laboratory type—was feeding and free to move about in the chamber, so that the figures were not for basal metabolism, but they covered at least 21 hours for all days throughout the experiment.

TABLE I Rabbit 2 (2.8 kilos) average figures

$O_2$ pressure in inspired air mm. Hg	$O_2$ consumption c.c. per min	Duration days	Temp in chamber ° C
144	28.5	12	23.3
85	26.2	19	22.5
71	30.3	14	22.9
53	27.1	10	22.6
143	32.3	11	21.1

There did appear to be a slight rise in  $O_2$ -consumption as an after-effect of the exposure, which persisted for some days, but eventually the  $O_2$ -consumption fell somewhat below the original level. On expeditions to high altitudes little change has been recorded in metabolism of man (Haldane and others(6), Barcroft(7)). Occasionally a rise has been reported, but this is usually regarded as an effect of exposure to cold.

Prolonged exposure of rabbits to high  $O_2$ -pressure in the inspired air was also without effect upon the metabolism (Fig 3 and Table II) This is in agreement with results obtained for a few hours' exposure(5) The figures in the table are not for basal metabolism, but cover practically the entire period of the experiment

TABLE II. Rabbit 3 (3.4 kilos), average figures

$O_2$ pressure in inspired air mm Hg	$O_2$ consumption c.c per min.	Duration days	Temp in chamber ° C.
141	31.7	7	22.3
291	30.4	18	23.5
416	30.1	20	22.2
160	29.5	9	23.2

*Weight* There was a gradual loss of weight during exposure to lowered  $O_2$ -pressure in the air with a tendency to return towards the original figure. The maximum loss was about 12 p.c. during exposure to  $O_2$ -pressure at about 53 mm Hg (Fig 2), loss of weight was made good after the return to normal air, but later the animal lost some weight again, loss of weight in man is a common effect of exposure to high altitude. Similarly exposure to high  $O_2$ -pressure in the air caused a maximum loss of about 12 p.c. in weight (see Fig 3), after the return to normal  $O_2$ -pressure the animal's weight gradually returned to normal.

*Hb and red corpuscles* Prolonged exposures to altered  $O_2$ -pressures in the air had profound effects upon the blood. The main results indicated that the Hb p.c. and the red cell count increased as the pressure in the air diminished, and *vice versa*, and that the functions of the Hb-controlling organs were regulated by the  $O_2$ -content of the blood. The important facts from the experiments illustrated in Figs 2 and 3 are grouped together in Table III and Fig 4.

TABLE III.

$O_2$ pressure in inspired air mm Hg	Red cells millions per c mm	Hb p.c.	Colour index	Rabbit
53	9.5	120	0.77	2
71	9.0	115	0.78	
85	7.5	105	0.86	
144	5.5	90	1.00	
420	3.0	60	1.22	
79	7.7	100	0.92	3
141	6.0	85	1.00	
291	4.2	72	1.21	
416	3.0	55	1.29	

The experiment with high  $O_2$ -pressure using Rabbit 2 was commenced 108 days after the end of the experiment with low  $O_2$ , the

exposure to high  $O_2$  lasting 4 weeks, and the low  $O_2$ -pressure experiment with Rabbit 3 was carried out 56 days after the high  $O_2$ -experiment

It has long been known that a lowered  $O_2$ -pressure in the air eventually increases the Hb p c and the red cell count independently of barometric pressure. The opposite that exposure to  $O_2$ -pressures above normal decreases the Hb p c and the red cell count has not been demonstrated, so far as I know, independently of barometric pressure. Haldane(8) did infer that this would be the case and Adele Bornstein(9) demonstrated that prolonged exposure to increased pressure of air (+2 atmospheres) in the Elbe tunnel decreased the Hb p c and the red cell count by about 20 p c in dogs and a monkey, but she did not determine whether this was due to the high barometric pressure or to the high  $O_2$ -pressure, my experiments proved that the latter was the case. Adele Bornstein thought that the changes in the Hb were due to a mere dilution of the blood, although the evidence put forward was by no means convincing. The question whether the changes in Hb and in red cells are, or are not, due to simple alterations in the concentration of the blood has been reviewed by Haldane(10) and by Barcroft(11), both of whom hold that there is an absolute increase in the Hb-content of the blood on exposure to lowered  $O_2$ -pressure and not a mere concentration of the blood. My results agreed with their view. Thus the changes observed in Hb p c and in the red cell count were not at all in proportion, as they should have been if there had been a simple concentration or dilution of the blood. I found that as the  $O_2$ -pressure in the air increased the colour index—or the content of Hb in each corpuscle—also increased (Fig 4 and Table III). It is of interest to note that the colour index during lowered  $O_2$ -pressure resembled that of chlorosis, whilst during increased  $O_2$ -pressure it resembled that of pernicious anaemia, the change in  $O_2$ -pressure, however, did not alter the types nor the size of the red cells, the reticulated red cells were estimated and numbered 8 per 1000 after 4 weeks' exposure to high  $O_2$ -pressure in the air and 80 per 1000 10 days after return to normal  $O_2$ -pressure, so that on the evidence of this one experiment formation of red cells was retarded by increased pressure of  $O_2$ , in another experiment 11 days' exposure to low  $O_2$ -pressure (79 mm Hg) increased the reticulated red cells from 30 to 75 per 1000.

That there was more than a simple alteration in the concentration of the blood during exposures to altered  $O_2$ -pressure was supported also by the time required to establish the changes in Hb p c and red cell count and to re-establish the normal conditions after resumption of

normal  $O_2$ -pressure in the air, several weeks being required in each case. Other factors indicating an absolute change in the Hb-controlling organs were the after-effects upon the  $O_2$ -tensions in the tissues which could be explained by definite alterations in the amounts of Hb in the blood, as we shall see later.

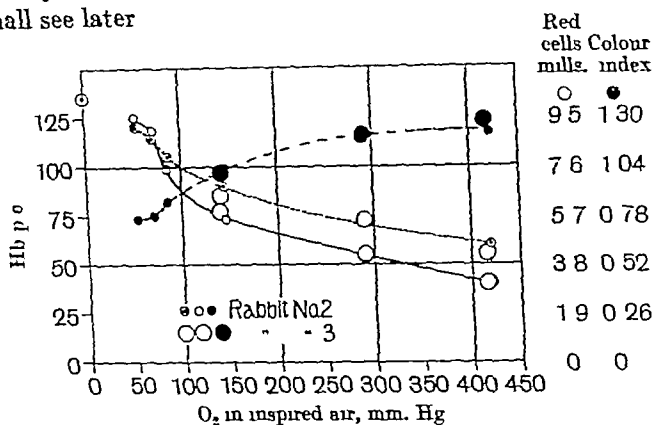


Fig. 4 Relations between Hb p c, red cell count, colour index and  $O_2$  pressure in the air during prolonged exposures.

The curves for Hb p c and red cell count when plotted against  $O_2$ -pressures in the inspired air (Fig. 4) exhibit a gradual bend in the region below 150 mm pressure, this is perhaps evidence of a mass action effect in the sense that a change from 50 to 100 mm has about as much effect as a change from 100 to 200 mm or from 200 to 400 mm.

*White corpuscles* With Rabbit 2 there was an increase in white cells from about 7250 to 10,250 per c mm as the result of exposure both to lowered  $O_2$ -pressure in the air (Fig. 2) and to increased  $O_2$ -pressure, on the other hand, Rabbit 3 exhibited no change in the white cell count on exposure to high or to low  $O_2$ -pressure. The effects upon the white cell count were therefore very variable.

The differential counts exhibited rather more definite changes, lowered  $O_2$ -pressure (53 mm Hg) increased the polymorpho-nuclear cells, whilst increased  $O_2$ -pressure (416 mm Hg) decreased these cells (Table IV). These changes, however, were not easily reversed, persisting for weeks after return to normal  $O_2$ -pressure. The blood samples were taken about 11.0 a.m. from the vein of an ear rendered hyperæmic by heat, and the figures refer to the last days of each period of exposure to different  $O_2$ -pressures. Haldane and his co-workers (12) observed in rabbits and in other subjects that the lymphocytes were increased at an altitude with about 85 mm Hg pressure of  $O_2$  in the air.

exposure to high  $O_2$  lasting 4 weeks, and the low  $O_2$ -pressure experiment with Rabbit 3 was carried out 56 days after the high  $O_2$ -experiment

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That there was more than a simple alteration in the concentration of the blood during exposures to altered  $O_2$ -pressure was supported also by the time required to establish the changes in Hb p c and red cell count and to re-establish the normal conditions after resumption of

normal  $O_2$ -pressure in the air, several weeks being required in each case. Other factors indicating an absolute change in the Hb-controlling organs were the after-effects upon the  $O_2$ -tensions in the tissues which could be explained by definite alterations in the amounts of Hb in the blood as we shall see later.

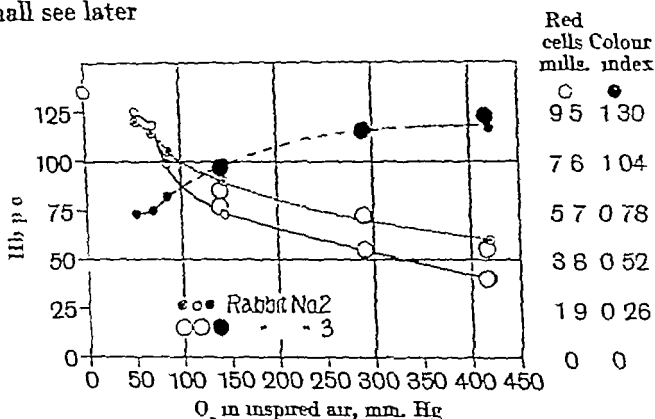


Fig 4 Relations between Hb p.c., red cell count, colour index and  $O_2$  pressure in the air during prolonged exposures

The curves for Hb p.c. and red cell count when plotted against  $O_2$ -pressures in the inspired air (Fig 4) exhibit a gradual bend in the region below 150 mm. pressure, this is perhaps evidence of a mass action effect in the sense that a change from 50 to 100 mm. has about as much effect as a change from 100 to 200 mm. or from 200 to 400 mm.

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I found a similar change in one case with pressures as low as 71 but not lower, the lymphocyte elements, as already stated, being decreased by very low  $O_2$ -pressures and increased by very high  $O_2$ -pressures

TABLE IV White corpuscle percentages

$O_2$ pressure in inspired air mm. Hg	Polymorphs	Small lymphocytes	Large lymphocytes	Large mononuclears	Rabbit
144	24.7	62.5	4.8	8.0	2
85	27.0	64.0	3.5	5.5	
71	16.2	66.5	8.1	9.2	
53	44.0	46.6	1.6	7.8	
146	47.4	47.4	0.5	4.7	
420	18.8	64.1	9.2	7.9	
141	20.1	70.9	5.4	3.6	3
291	26.5	65.4	2.6	5.5	
416	10.5	75.3	3.1	11.1	
146	7.4	70.1	6.8	15.7	
77	39.0	40.2	10.9	9.9	

*CO<sub>2</sub>-tensions in the tissues* The  $CO_2$ -tensions in the tissues on the whole increased as the  $O_2$ -pressure in the air increased and were controlled chiefly by the  $O_2$ -content of the blood. Lowered  $O_2$ -pressure in the inspired air caused a marked fall in  $CO_2$ -tensions in the tissues (Figs 2 and 5, and Table V), whilst increased  $O_2$ -pressure caused a slight but apparently definite rise in  $CO_2$ -tensions (Figs 3 and 5, and Table V), these results confirm a previous research (5), in which rabbits were exposed for a few hours to altered  $O_2$ -pressures. From the graphs in Figs 2 and 3 it will be observed that constancy had become established, since the curves for  $CO_2$ -tensions are practically straight lines in each period.

TABLE V  
 $CO_2$  tensions in tissues  
mm. Hg

$O_2$ pressures in inspired air mm. Hg	mm. Hg		Rabbit
	Skin	Abd. cav.	
53	20	19	2
71	24	22	
85	33	31	
144	47	46	
320	50	48	
420	50	48	
80	24	25	3
141	38	39	
291	43	44	
416	44	47	

As with the curves for Hb p c and the red cell count the curves for  $CO_2$ -tensions in the tissues when plotted against  $O_2$ -pressure in the air (Fig 5) exhibit a marked shoulder in the region of normal  $O_2$ -pressure in the air (about 150 mm. Hg), the curve being much steeper at the low

values for  $O_2$ -pressures than at the high values. The respiratory centre is stimulated by a low supply of  $O_2$ , so that  $CO_2$  is removed from the

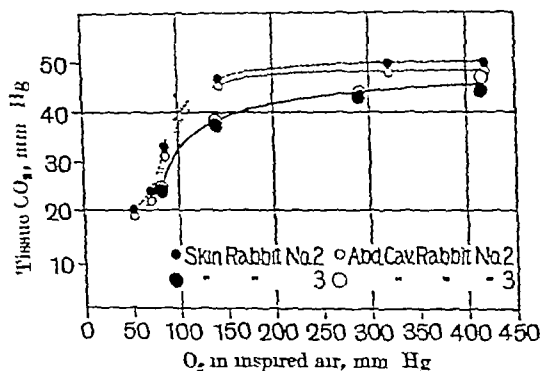


Fig. 5 Relations between  $CO_2$  tensions in the tissues and  $O_2$  pressure in the air during prolonged exposure

tissues and the  $CO_2$ -tensions in the tissues fall, on the other hand, the respiratory centre is depressed by too great a supply of  $O_2$  at high  $O_2$ -pressures in the air and the  $CO_2$ -tension rises in the tissues. It is suggested that  $O_2$ -supply is Respiratory X of Yandell Henderson (13), by  $O_2$ -supply is meant both  $O_2$ -deficiency and  $O_2$ -excess. Y Henderson showed that  $O_2$ -deficiency in itself could not explain all the facts but  $O_2$ -excess appears to supply the necessary missing link, further evidence will be given later on in this paper, that the body strenuously opposes excess of  $O_2$  in the tissues.

Many factors have been credited with a controlling influence upon the  $CO_2$ -tensions in the alveolar air and in the tissues during alterations in  $O_2$ -pressure in the air, it is generally agreed that lowered  $O_2$ -pressure in the air increases the sensitivity of the respiratory centre to  $CO_2$ , thus increasing ventilation of the lung (Barcroft and others (14)), Yandell Henderson (15) showed that blood alkali is decreased by low  $O_2$ , so that  $CO_2$  must be removed to keep the pH normal, again, lowered  $O_2$ -tension alters the  $CO_2$ -dissociation curve of blood, so that  $CO_2$  would be more easily removed from the tissues (Christiansen, Douglas and Haldane (16)), also Hb carries almost half the  $CO_2$  in normal blood and more if the venous blood is completely reduced (Joffe and Poulton (17)), Hb also acts as an acid and when Hb is increased this effect would also be increased. On the other hand, with increased  $O_2$ -pressures in the air, we should expect the opposite effects. In either case the ventilation of the lung is probably the chief factor controlling  $CO_2$ -tensions in the tissues.



Somervell(18), during the Mount Everest expedition, found the alveolar  $\text{CO}_2$ -tension to be about 20 mm Hg at 16,500 feet, and about 8 mm Hg at 23,000 feet in place of the normal figure of about 38 mm Hg, the figure for alveolar  $\text{CO}_2$  at 16,500 feet fits in fairly well with those for the tissue  $\text{CO}_2$ -tensions, but that for 23,000 feet seems rather low, although I have found considerable differences between arterial  $\text{CO}_2$ -tensions and tissue  $\text{CO}_2$ -tensions(19) in resting animals, these low results of Somervell have been criticised because he used rubber bags to hold the samples

*O<sub>2</sub>-tensions in the tissues* The  $\text{O}_2$ -tensions in the tissues increased as the  $\text{O}_2$ -pressure in the air increased, being controlled by the  $\text{O}_2$ -content of the blood. As with the  $\text{CO}_2$ -tensions in the tissues the curves for the  $\text{O}_2$ -tension in the tissues when plotted against the  $\text{O}_2$ -pressure in the inspired air (Fig 6) exhibit a marked shoulder in the region below the normal atmospheric  $\text{O}_2$ -pressure (150 mm Hg), the steepest part of the curves appearing at the region where the  $\text{O}_2$ -content of the blood is most altered, that is, at low  $\text{O}_2$ -pressure in the air (see Fig 7)

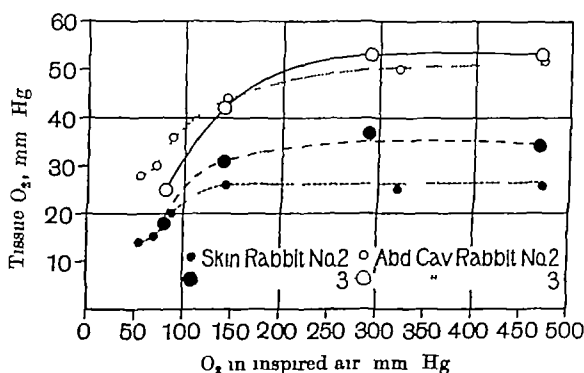


Fig 6 Relations between  $\text{O}_2$  tensions in the tissues and  $\text{O}_2$  pressure in the air during prolonged exposures

The details of the chief experiment with lowered  $\text{O}_2$ -pressure in the air are given in Figs 2 and 6, and Table VI, those of the chief experiment with high  $\text{O}_2$ -pressure are given in Figs 3 and 6, and Table VI. It will be noted that in the later experiment (Fig 3), although the  $\text{O}_2$ -tension in the abdominal cavity was quite definitely increased throughout the whole experiment, the  $\text{O}_2$ -tension under the skin was only increased for the first days, afterwards falling towards normal, this was probably due to local alterations in circulation, e.g. slowing by constriction of vessels

In this experiment, during exposure to  $O_2$ -pressure at 420 mm Hg in the air, the  $O_2$ -tension under the skin exhibited marked oscillations (Fig 3) for a time, probably owing to constriction and relaxation of vessels, this was not observed in the other experiment at the same pressure and, moreover, it is obvious from the graphs in Figs 2 and 3 that, on the whole, constancy for  $O_2$ -tensions in the tissues had become established in each period of exposure

The relations between tissue  $O_2$ -tensions and Hb p c between tissue  $O_2$ -tensions and tissue  $CO_2$ -tensions and also between tissue  $CO_2$ -tensions and Hb p c were studied. There were close relationships in all cases, at least during exposures to low  $O_2$ -pressures in the air but the relations could not be represented by continuous straight lines over all alterations in  $O_2$ -pressures

TABLE VI.

$O_2$ pressures in inspired air mm. Hg	$O_2$ tensions in tissues mm. Hg		Rabbit	Condition of animal
	Skin	Abd. cav		
53	14	28	2	Acclimatised
71	15	30		
85	20	36		
144	26	44		
320	25	50		
420	26	52	3	First days of exposure
80	18	25		
141	31	42		
291	41	58		
416	42	70		

In the experiments with lowered  $O_2$ -pressure it is of interest to note at what levels were the  $O_2$ -tensions in the tissues when symptoms of ill-effects, *e.g.* tetany, developed. In one unacclimatised animal marked tetany occurred when the  $O_2$ -tension in the abdominal cavity had fallen from 40 to 22 mm. Hg and that under the skin from 25 to 11 mm. Hg, this animal died. In another animal—somewhat acclimatised—symptoms of tetany (slight tremors) developed when the  $O_2$ -tension in the abdominal cavity had fallen from 44 to 28 mm. Hg and that under the skin from 26 to 14 mm. Hg (Fig 2). The process was slow and insidious, and signs of tetany did not occur for days after the tensions had fallen to these levels, indicating that such low tensions could be tolerated for some days without development of tremors and other ill-effects. However, it was obvious that there was a limit to the period for safety during exposure to  $O_2$ -pressures in the air as low as 53 mm. Hg ( $7\frac{1}{2}$  p c), and with the  $O_2$ -tension in the abdominal cavity about 25 mm. Hg, rabbits might survive for periods as long as ten days but even if they

had been acclimatised previously to less severe degrees of  $O_2$ -deficiency, they were in danger from tetany. These results are of interest in connection with Mount Everest, where the  $O_2$ -pressure is about 53 mm Hg, from the dissociation curve it has been concluded that this figure for inspired air must be near the danger point for man, because the blood carries only sufficient  $O_2$  for fairly quiet conditions or for resting metabolism.

It was observed (Figs 2 and 6) that, when the  $O_2$ -pressure in the inspired air had been lowered below 70 mm Hg, the rate of decrease in the  $O_2$ -tensions in the tissues became less, probably indicating that the body mechanisms were opposing a further fall, since symptoms of tetany developed at these low pressures, had the tissue  $O_2$ -tensions fallen much further—that is, below 25 mm Hg in the abdominal cavity—death probably would have occurred, the curves for tissue  $CO_2$ -tensions (Fig 5) also exhibit a retardation at very low  $O_2$ -pressures. These retardations would be expected in any case from the shape of the  $O_2$ -dissociation curve (see Fig 7).

It was an easier matter by decrease of ventilation, etc., to keep out  $O_2$  from the tissues when exposed to increased  $O_2$ -pressure in the air than to bring in  $O_2$  into the tissues by increase of ventilation, etc., when exposed to lowered  $O_2$ -pressure in the air. Thus prolonged increase (by 196 p c) of  $O_2$ -pressure in the air only raised the  $O_2$ -tension in the abdominal cavity by 26 p c and scarcely at all under the skin (Fig 3), whereas prolonged decrease (by 63 p c) of  $O_2$ -pressure in the air lowered the  $O_2$ -tension in the abdominal cavity by 36 p c and under the skin by about 46 p c (Fig 2). The main reason for this is the shape of the  $O_2$ -dissociation curve (see Fig 7), at high  $O_2$ -pressure in the air the change in  $O_2$ -content of the blood being very small and due only to the increase in amount of  $O_2$  in solution, acclimatisation as will be seen later has some influence in the matter.

In the high  $O_2$ -pressure experiments the animal endeavoured to prevent the  $O_2$ -tension in the abdominal cavity remaining much above 50 mm Hg, in the low  $O_2$ -pressure experiments this tension was kept at about 25 mm Hg, 25–50 mm would thus appear to be the limits for safety.

The interposition of single "control" days with normal  $O_2$ -pressure in the air during the course of both experiments, illustrated in Figs 2 and 3, gave confirmation to the results described above, that is, the  $O_2$ - and  $CO_2$ -tensions in the tissues increased as the  $O_2$ -pressure in the inspired air increased, and these effects were observed almost imme-

diately previous experiments (5) for short periods of exposure to altered  $O_2$ -tensions gave similar results

*After-effects of prolonged exposure to lowered  $O_2$ -pressure* In the experiment illustrated in Fig 2 the  $O_2$ -pressure in the air was raised to normal again after 43 days of exposure to lowered  $O_2$ -pressure, important after-effects concerned the red corpuscles, the Hb and the  $CO_2$ - and  $O_2$ -tensions in the tissues, indicating that the animal was now un-acclimatised to the normal  $O_2$ -pressure in the air. The red cell count and the Hb p.c. did not return from their high levels to normal for several weeks indicating that the Hb controlling organs suffered more than a

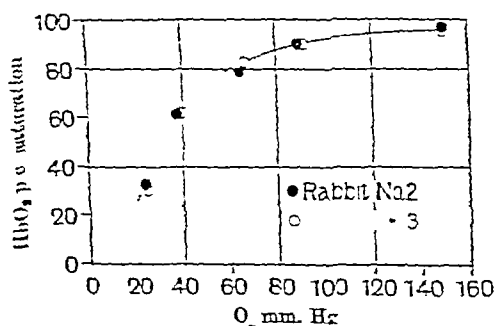


Fig 7  $O_2$ -dissociation curve of blood of Rabbits Nos. 2 and 3 at 37 mm. Hg pressure of  $CO_2$ . Hb p.c. = 75-78.  $O_2$ -content of arterial blood = 12.5 vols. p.c. or about 90 p.c. saturation. Observations taken about four weeks after completion of  $O_2$ -pressure experiments.

temporary change, in other words, the production of red cells had evidently been stimulated and time was required before the normal conditions could be re-established.

The  $CO_2$ -tensions in the tissues did return towards normal but remained below normal level for some weeks (Fig 2 and Table VII), probably because ventilation of the lung remained above normal owing

TABLE VII.

$O_2$ pressure in inspired air mm. Hg	$O_2$ tensions in tissues mm. Hg		Hb p.c.	$CO_2$ tensions in tissues mm. Hg		Time
	Skin	Abd. cav.		Skin	Abd. cav.	
144	20	44	90	47	46	Normal figures, immediately before exposure to low $O_2$
75	14	38	120	21	21	Last day of exposure to low $O_2$
14	40	55	120	35	33	Day after return to normal $O_2$

to the respiratory centre retaining for some time its increased sensitivity to  $\text{CO}_2$ , both the decreased alkali of the blood and the increased Hb which acts as an acid would require time to return to normal, the curves for  $\text{CO}_2$ -tensions and for Hb exhibited much the same time relations in their gradual return to normal (Fig 2)

The  $\text{O}_2$ -tensions in the tissues returned to normal immediately and increased to far above normal (Fig 2 and Table VII) owing to the marked increase in Hb, the curves for Hb and for  $\text{O}_2$ -tensions in the tissues exhibited much the same time relations, returning to normal levels gradually (Fig 2)

*After-effects of prolonged exposure to increased  $\text{O}_2$ -pressure* In the experiment in Fig 3 the exposure to high  $\text{O}_2$ -pressure in the air lasted in all for 38 days, interesting after-effects were observed as the result of sudden return to normal  $\text{O}_2$ -pressure in the air again, revealing that the animal was unacclimatised now for normal pressure. The first effect noticed was hyperpnœa, total ventilation of the lung was not recorded accurately, but observation of the rate of breathing (30 p c increase) and of the movements of the chest and abdomen indicated definite hyperpnœa. At the same time the  $\text{CO}_2$ -tensions in the tissues were markedly decreased, that in the abdominal cavity falling from 47 to 30 mm Hg and that under the skin from 44 to 33 mm Hg (Fig 3 and Table VIII), the normal  $\text{CO}_2$ -tensions for this animal being about 38 mm Hg. The  $\text{CO}_2$ -tensions remained at a low level for over two weeks. This time the  $\text{O}_2$ -tensions in the tissues of Rabbit 3 had fallen from 53 to 32 mm Hg (a 40 p c fall), whilst that under the skin had fallen from 35 to 25 mm Hg (Fig 3 and Table VIII), the normal figures being 43 and 32 respectively.

TABLE VIII Rabbit 3

$\text{O}_2$ pressure in inspired air mm Hg	$\text{O}_2$ tensions in tissues mm Hg		Hb p c	$\text{CO}_2$ tensions in tissues mm Hg		Time
	Skin	Abd cav		Skin	Abd. cav	
141	32	43	85	38	39	Normal figures immediately before exposure to high $\text{O}_2$
416	35	53	55	44	47	Last day of exposure to high $\text{O}_2$
147	25	32	55	33	30	Day after return to normal $\text{O}_2$

In a similar experiment with Rabbit 2, the  $\text{O}_2$ -tension under the skin fell 44 p c below normal and that in the abdominal cavity 25 p c below normal. The fall was due to the low Hb p c, since the curves for Hb and for  $\text{O}_2$ -tensions on the whole followed one another in their time

relations during their gradual return to normal (Fig 3) The exposure of the animal to an increase of 200 p c in  $O_2$ -pressure in the air had obviously altered its whole condition At this high pressure the  $O_2$ -tension in the abdominal cavity had become constant at the high level of 53 mm Hg—the normal figure being 43—and it is likely that a similar increase in  $O_2$ -tension prevailed in the other central organs, *e g* brain, spinal cord, so that these tissues had now become acclimatised to this high  $O_2$ -tension, and owing to the fall in Hb they were no longer able to function normally on lowering the  $O_2$ -pressure in the air to normal again Increased breathing was necessary, hence the hyperpnoea and the great fall in  $CO_2$ -tension in the tissues The animal was just as handicapped in its endeavours to obtain  $O_2$  from normal air as was the normal Rabbit 2 when first exposed to air with  $O_2$  lowered to 85 mm. Hg (Fig 2)

*Factors in acclimatisation to lowered  $O_2$ -pressure in the air* Haldane<sup>(20)</sup> holds that secretion of  $O_2$  by the lungs is necessary to explain rapid acclimatisation Barcroft<sup>(21)</sup> opposes this theory and he<sup>(22)</sup> enumerates several factors such as increased ventilation, increased Hb, change in the dissociation curve<sup>(23)</sup> and vascular adjustments in the medulla Both observers favour the view that in acclimatisation the tissue  $O_2$ -tension is kept near normal in accordance with Claude Bernard's conception of the maintenance of the normal environment for the cell

My experiments with prolonged exposure to low  $O_2$ -pressure give no support to the theory that the  $O_2$ -tensions in the tissues are improved greatly by acclimatisation The  $O_2$ -tensions in the tissues fell markedly on the first day of low  $O_2$ -pressure, and there was no evidence of material improvement in tissue  $O_2$ -tension as each period of exposure to low  $O_2$  became extended (Fig 2 and Table IX) The normal environment for the cell was therefore not re-established, and this is probably why individuals are not the same at high altitudes as at sea-level.

TABLE IX. Rabbit 2

$O_2$ pressure in inspired air mm. Hg	$O_2$ tensions in tissues mm. Hg		Time
	Skin	Abd. cav	
144	26	44	Normal figures before and after experiment of exposure to low $O_2$
85	19	34	2nd day of exposure to 1st period of low $O_2$
85	20	36	19th day of exposure to 1st period of low $O_2$
71	13	29	1st day of exposure to 2nd period of low $O_2$
71	15	29	14th day of exposure to 2nd period of low $O_2$
53	14	28	1st day of exposure to 3rd period of low $O_2$
53	14	28	10th day of exposure to 3rd period of low $O_2$

The chief factor in rapid acclimatisation is the ability of the vital tissues (heart, medulla, respiratory organs) to tolerate a lowered  $O_2$  tension and to function normally, we know that normally the blood carries about three times the necessary quantity of  $O_2$  for resting metabolism, so there should be a sufficiency of  $O_2$  if the tissue can tolerate the lowered  $O_2$ -tension. In the later stages of acclimatisation increase of Hb appears to be the chief factor.

*Factors in acclimatisation to increased  $O_2$ -pressure in the air* The possible or probable factors are decreased ventilation, change in dissociation curve, vascular changes (Haldane(24)) and decrease of Hb. Ventilation of lung was not accurately recorded in these prolonged experiments, but it was recorded in short experiments, a decrease of 30 p c being observed whilst breathing  $O_2$  at the increased pressure specified. Decrease of Hb was marked in the present experiments, but even a fall of 35 p c in Hb and 50 p c in red cell count did not bring the  $O_2$ -tension in the abdominal cavity down to normal again (Fig 3 and Table X), so that here also the normal environment of the cell was not re-established.

TABLE X Rabbit 3

$O_2$ pressure in inspired air mm. Hg	$O_2$ tensions in tissues mm. Hg		Time
	Skin	Abd. cav	
141	31	42	Normal figures before and after experiment of exposure to high $O_2$
291	41	58	2nd day of exposure to 1st period of high $O_2$
291	37	53	18th day of exposure to 1st period of high $O_2$
416	42	70	3rd day of exposure to 2nd period of high $O_2$
416	35	53	20th day of exposure to 2nd period of high $O_2$

It is obvious from these great decreases in Hb and red cells that the body strenuously opposes excess  $O_2$  in the tissues. Administration of  $O_2$  therapeutically or at high altitudes can be of no value unless there is an actual deficiency of  $O_2$  under the existing conditions. The changes in Hb, and in  $O_2$ - and  $CO_2$ -tensions in the tissues during and after exposure to greatly lowered  $O_2$ -pressure indicate that the body is completely changed by acclimatisation, and administration of  $O_2$  to individuals acclimatised to very high altitudes is obviously not the same thing as at sea-level. This probably explains the failure of administration of  $O_2$  during the last Everest expedition.

*Control of tissue  $O_2$ -tension* Amongst the possible or probable controlling factors may be mentioned  $O_2$ -pressure in the air, quantity of Hb, circulation rate and vascular adjustment, diffusion and secretion

through the lung, and through the capillary wall in the tissues, and  $O_2$ -metabolism

From the evidence in this paper the important factors are the  $O_2$ -pressure in the inspired air and the Hb (Fig 6 and Tables VII and VIII)  $O_2$ -metabolism appears to be independent of tissue  $O_2$ -tension, since the  $O_2$ -consumption remained constant whilst the tissue  $O_2$ -tensions were markedly changed. Further,  $O_2$ -consumption and metabolism may be greatly changed without affecting the  $O_2$ -tensions in the tissues, this was demonstrated in four different experiments in which rabbits were fed with thyroid extract (1.3 gm per diem, Burroughs and Wellcome "Tabloid") for four weeks. Such excessive thyroid feeding caused a marked increase in consumption of food and a marked loss of weight, but the  $O_2$ - and  $CO_2$ -tensions in the tissues remained unaltered (Table XI)

TABLE XI. Rabbit

Time (days)	Conditions	Weight gm.	$O_2$ tensions in tissues mm. Hg		$CO_2$ tensions in tissues mm. Hg	
			Skin	Abd. cav	Skin	Abd. cav
0	Normal	2450	23	41	49	51
3	Thyroid feeding	2450	24	38	51	51
17	"	1870	27	38	52	51
29	"	1565	27	40	52	52

In all the experiments completed, when the after-effects due to the alterations of  $O_2$ -pressure in the air had subsided, the  $O_2$ -tensions in the tissues returned to their original normal level. These tensions as measured are considered to exist immediately outside the cell wall of the particular tissue, probably they resemble closely the tensions in the venous blood, how closely it is not possible to say.

#### SUMMARY

(1) Rabbits were exposed for prolonged periods (about six weeks) to lowered  $O_2$ -pressure in the air, as low as 60 p.c. below normal, and to increased  $O_2$ -pressure, up to 200 p.c. above normal, the barometric pressure being kept at 745–760 mm Hg. Such alterations in  $O_2$ -pressure—produced more or less gradually—had no effect upon the  $O_2$ -consumption.

(2) Prolonged exposure to lowered  $O_2$ -pressure depressed the  $CO_2$ - and  $O_2$  tensions in the tissues to a marked degree and markedly increased the Hb and red cells. There were marked after-effects upon all these factors, indicating the existence of mechanisms concerned with acclimatisation.



(3) Prolonged exposure to increased  $O_2$ -pressure slightly increased the  $CO_2$ -tensions in the tissues and quite definitely increased the  $O_2$ -tension in the abdominal cavity, the Hb and the red cells were markedly decreased. Marked after-effects connected with acclimatisation were also produced.

(4) The main factor in rapid acclimatisation to lowered  $O_2$ -pressure in the air is the ability of the vital organs (heart, respiratory organs, medulla) to withstand a surrounding low  $O_2$ -tension in their immediate environment and to maintain their normal supply of  $O_2$ . There is no evidence that acclimatisation raises the tissue  $O_2$ -tension to anywhere near normal again, so that, contrary to Claude Bernard's conception, the normal environment of the cell is not re-established, although undoubtedly the  $pH$  is kept almost constant, increase of Hb which occurs at a later stage is a factor in the later stages of acclimatisation, probably relieving the vital organs in their efforts to supply the necessary  $O_2$ .

(5) Rabbits—previously acclimatised to some degree—tolerated  $7\frac{1}{2}$  p c (53 mm Hg)  $O_2$ -pressure in the air for as long as 10 days, but signs of tetany then became apparent, the  $O_2$  at Mount Everest is about  $7\frac{1}{2}$  p c.

(6) Owing chiefly to the shape of the  $O_2$ -dissociation curve the organism is better able to counteract a high  $O_2$ -pressure in the air than a low pressure, nevertheless, under the former condition the  $O_2$ -tension in the tissues, at least in the central organs, is much above normal even after acclimatisation for six weeks, so that here also the normal environment of the cells is not completely re-established. The body exerts marked efforts to keep out excess of  $O_2$  from the tissues.

(7) Decrease of Hb and of red cells is an important factor in the later stages of acclimatisation to increased  $O_2$ -pressure in the air. The Hb fell from 85 to 55 p c and the red cells from 60 to 30 millions per c mm, the animals exhibited hyperpnœa and a marked fall of  $CO_2$ -tensions in the tissues—indicating  $O_2$ -deficiency—on reducing the  $O_2$ -pressure in the air to normal again. The  $O_2$ -supply is an important factor controlling respiration, excess of  $O_2$  depressing the centre and an inadequate supply of  $O_2$  stimulating the centre, it is suggested that  $O_2$ -deficiency and  $O_2$ -excess taken together constitute Respiratory X of Henderson. The  $CO_2$ -tensions in the tissues are regulated chiefly by the  $O_2$ -content of the arterial blood, they increase as the  $O_2$ -pressure in the inspired air increases.

(8) The function of the Hb controlling organs is regulated chiefly by the  $O_2$ -content of the blood, the Hb and the red cells decreasing as the  $O_2$ -pressure in the inspired air increases, considerable time is required

for the establishment of these effects and for their reversals indicating more than a simple storage or other temporary change. The view that the alterations in Hb are due to changes in concentration of the blood is not supported. The colour index of the blood increases as the  $O_2$ -pressure in the air increases.

(9) The main factors controlling  $O_2$ -tension in the tissues are the  $O_2$ -pressure in the air in the lungs and the Hb, or in other words the  $O_2$ -content of the blood, the  $O_2$ -tensions in the tissues increase as the Hb and the  $O_2$ -pressure in the inspired air increase. The body resists a rise of tissue  $O_2$ -tension in the abdominal cavity to above 50 mm. Hg and a fall to below the level 25 mm. Hg.

(10) Excessive thyroid feeding has no effect upon the  $CO_2$ - and  $O_2$ -tensions in the tissues.

(11) It is suggested that lowering of Hb by exposure of an animal to increased  $O_2$ -pressure in the air may afford a method to test the action of drugs, etc., upon Hb formation.

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THE RATE OF DISTRIBUTION OF DISSOLVED GASES  
BETWEEN THE RED BLOOD CORPUSCLE AND  
ITS FLUID ENVIRONMENT Part I Preliminary  
experiments on the rate of uptake of Oxygen and  
Carbon Monoxide by sheep's corpuscles

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INTRODUCTION

DURING the past three years we have been engaged in applying our method of measuring the velocity of very rapid chemical reactions<sup>(1, 2)</sup> to the investigation of the kinetics of the reactions between hæmoglobin and dissolved gases<sup>(3, 4, 5, 6)</sup> The quite unexpected success, which has attended these researches, has encouraged us to direct our inquiries to a problem with more pronounced physiological implications, namely, that of determining the rate of distribution of dissolved gases between the red blood corpuscle and its fluid environment The interest of this problem is not far to seek It has hitherto been tacitly assumed in many quarters, though as far as we know without any adequate experimental basis, that the rate of distribution of dissolved oxygen between the corpuscle and the plasma is so rapid as to have no appreciable effect in limiting the rate of oxygen distribution within the body, and that the latter is solely determined by the pressure gradient between the alveolar air and the plasma (for external respiration) and between the plasma and the interior of the tissue cell (for internal respiration) Even if direct measurements of the rate of distribution confirm this tacit assumption, it would still be of great interest to know what factors condition the observed rate of distribution For example, which is of predominating importance, the rate of diffusion of dissolved gas through the corpuscle membrane or the rate of the chemical reaction inside the corpuscle? It would also be of interest to know to what extent these factors are affected by changes in other variables such as temperature, pH, salt concentration, ion-balance, subminimal doses of laking agents, etc

It must be emphasised at the outset that the *in vitro* experiments to be described below cannot furnish a legitimate basis for deductions as to the rate of gas distribution inside the living blood capillary, unless it has previously been shown (a) that the concentration of dissolved gases at any instant of time is uniform in all parts of the fluid surrounding the corpuscles under investigation<sup>1</sup>, (b) that the condition of the corpuscles, at any rate as regards the particular property here being studied, is not affected by the treatment which they receive prior to the *in vitro* experiment. The control experiments necessary to show that requirement (a) is properly satisfied have been simple to carry out and seem to us to be quite conclusive. In regard, however, to requirement (b) it has been necessary, not only to devise new and more elaborate apparatus, but also to work out to a finish the kinetics of the reactions between oxygen, carbon monoxide and hæmoglobin in solution. The difficulties involved in these last two problems have already delayed the completion of the present research for a very long time, and indeed seemed likely to delay it for some time to come. On this account it has seemed to us desirable to submit for publication an account of the preliminary successful experiments which we have already carried out. This paper contains (a) description of experimental details, (b) evidence that the suspension fluid surrounding the corpuscles in our experiments keeps uniformly mixed throughout, and finally (c) a brief preliminary account of the influence of several variables upon the rate of the process under investigation.

#### EXPERIMENTAL DETAILS

For studying the rate at which reduced corpuscles take up oxygen, a solution of 1 p c NaCl saturated with air at barometric pressure was mixed with a suspension of reduced corpuscles in 1 p c NaCl. The mixed suspension, after leaving the mixing chamber of the reaction velocity apparatus<sup>(1)</sup>, travelled with known speed down the observation tube, wherein determinations of the average percentage of  $O_2Hb$  in the corpuscles at various points were made by means of the Hartridge spectro-scope. The values of the latter, together with (i) the rate of linear flow, (ii) the total gas combining capacity of the hæmoglobin in the corpuscles, and (iii) the total amount of dissolved  $O_2$  per c c of suspension at the beginning of the process supply all the data required for quantitative treatment. The following experimental details require special mention.

<sup>1</sup> For reasons to be given in full in a later paper, it is highly probable that the concentration of dissolved  $O_2$  at all points within any given cross section of a blood capillary perpendicular to its length is uniform.

(a) *The reduced corpuscle suspension* 12 litres of warm 1 p c NaCl were evacuated in a 20-litre earthenware bottle, shaken by rolling and evacuated again, until all the  $O_2$  originally dissolved in the water was expelled. A quantity of sheep's blood, such as to give suitable spectroscopic bands in the subsequent examination of the suspension in the observation tube, was also reduced by warmth and evacuation, and then added anaerobically to the vacuous 12 litres of 1 p c NaCl. The two were then mixed by a few rapid shakes. Up to this point the technique was the same as that already used for the preparation of large quantities of reduced hæmoglobin solution(5). In order, however, to prevent the corpuscles by virtue of their higher specific gravity from settling to the bottom of the bottle during the course of the experiment and thus causing irregular readings in the observation tube, it was necessary to keep the bottle containing the corpuscle suspension continually rocked. This requirement precluded the direct application of compressed commercial nitrogen to the suspension, since this gas always contains sufficient oxygen as an impurity to re-oxygenate the corpuscles to a considerable extent. In order to cope with these difficulties the arrangement shown in Fig 1 was adopted. The details are sufficiently explained by the letter-press at the foot of the figure.

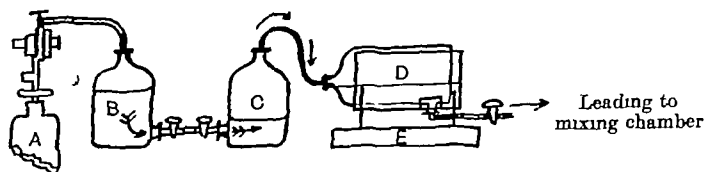


Fig 1 A=cylinder of compressed nitrogen. B=20 litre bottle filled initially with dilute alkaline hydrosulphite. C=20 litre bottle filled initially with chemically purified nitrogen. D=20 litre bottle half filled with reduced corpuscle suspension, the remaining space being initially vacuous, and afterwards replaced by pure  $N_2$ . E=rocking cradle

(b) *Possible laking of corpuscles* Although the suspension fluid employed (1 p c NaCl) was markedly hypertonic to sheep's blood corpuscles, it was feared that the violent handling experienced by the corpuscles in the mixing-chamber and the observation tube might cause appreciable hæmolysis. In order to find out whether any laking had taken place, portions of the effluent were set aside and allowed to stand for some hours. In all the experiments quoted in this paper, it was found that the corpuscles gradually settled, leaving a colourless layer of supernatant solution, thus proving that no laking had occurred. In a few

cases (especially if the temperature was above normal or if the blood used was not quite fresh), varying amounts of hæmolysis were noted. Experiments in which this occurred were discarded.

(c) *The determination of the p c  $O_2Hb$*  Preliminary observations showed that the reversion spectroscopic method used hitherto(4) for the determination of the p c  $O_2Hb$  in hæmoglobin solution, was also available even when the hæmoglobin was still enclosed in corpuscles. Owing, however, to the scattering of light by the highly refractile walls of the corpuscles, it was necessary to use a brighter source of light for the spectroscopic examination, to have the solid angle subtended at the spectroscopic slit larger, and also to have a higher concentration of blood in the suspension than in hæmoglobin solutions. The absorption bands were always more blurred in the case of the corpuscle suspension than in the case of hæmoglobin solutions, so that the accuracy of alignment of the bands and consequently of the p c  $O_2Hb$  determination were correspondingly reduced.

### EXPERIMENTAL RESULTS

The result of a typical experiment on a corpuscle suspension is shown by curve A in Fig 2

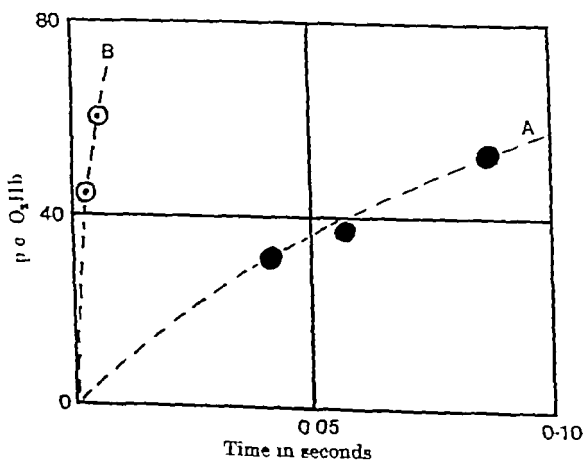


Fig 2 A = unlaked corpuscle suspension. B = laked corpuscle solution.

Curve B represents the average result obtained under otherwise similar conditions (e.g. same initial concentration of dissolved  $O_2$ , same gas combining capacity of hæmoglobin, same temperature, etc.) with a laked hæmoglobin solution. It will be observed that the rate of

increase of  $p_c \text{ O}_2\text{Hb}$  is about ten times smaller when the corpuscles are intact than when the corpuscles are laked

The discrepancy between the rates of the two processes may be *a priori* due to any of the following factors acting singly or in combination

(i) The existence of appreciable concentration gradients in the suspending fluid

The  $\text{O}_2$  which is acquired by the corpuscle must come, in the first instance, from the layers of suspending fluid adjacent to the corpuscles. The loss suffered by such layers will then be replenished by diffusion and by mixing with more distant layers. The latter two processes may be sufficiently slow to limit or at any rate to affect the rate of the actual process under investigation

(ii) The existence of an appreciable concentration gradient through the corpuscle membrane

We have as yet no certain quantitative information either as to the thickness or the diffusion coefficient of the corpuscle membrane. There is consequently a possibility that the  $\text{O}_2$  on the outside of the membrane may dam up, until there is a sufficient gradient through the membrane to enable  $\text{O}_2$  to enter the corpuscle at a rate equal to that at which it is removed by combination with the haemoglobin

(iii) The existence of an appreciable concentration gradient inside the corpuscle

The  $\text{O}_2$  which first enters the corpuscle will combine with the peripheral layer of Hb molecules. The next instalments of  $\text{O}_2$  will have to diffuse through the outer saturated layers in order to find Hb molecules which are still uncombined with  $\text{O}_2$ . It is possible that such diffusion may, as in (ii), be so slow as to produce an appreciable gradient of dissolved  $\text{O}_2$  inside the corpuscle

Any of factors (i), (ii), (iii) acting singly or in combination would involve a lower value of the average concentration of dissolved  $\text{O}_2$  in immediate contact with the Hb molecules in curve *B* of Fig. 2 than in curve *A*. There still, however, remains

(iv) The possibility that the kinetics of the reaction between  $\text{O}_2$  and Hb in the corpuscle may be so altered that even at the same average concentration of dissolved  $\text{O}_2$  the rate of increase of  $p_c \text{ O}_2\text{Hb}$  may be ten times smaller in the case of the corpuscle than in the case of the Hb solution

As mentioned in the Introduction, we have so far only been able to investigate factor (i) adequately

We shall now present in detail the evidence that the concentration gradients of dissolved  $O_2$  in the suspending fluid are negligible in size

The evidence required was obtained by means of the following control experiments

(i) *Variation of the rate of flow* The blank circles in Fig 3 were the values obtained at a rate of flow of 75 cm per sec down the observation tube, whereas the shaded circles represent the values obtained on the same corpuscle suspension at a rate of flow of 225 cm per sec. It will be seen that the blank circles and the shaded circles both fall (within experimental error) on the same curve. Now in heterogeneous processes which are limited by diffusion and mixing (7), the observed rate depends upon the rate of stirring, and indeed, in many cases, is approximately

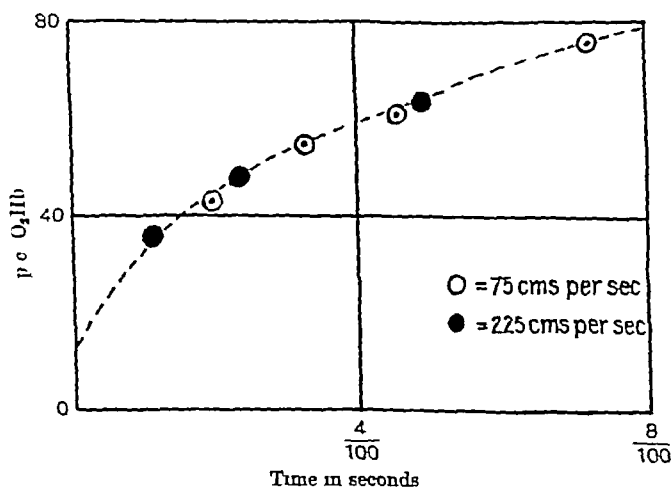


Fig 3

proportional to the  $\frac{1}{2}$ th power of the latter. Had diffusion plus mixing of the suspending fluid been the limiting factor here, the observed rate should in one case have been about  $\left(\frac{225}{75}\right)^{\frac{1}{2}}$ , i.e. 2.41 times the observed rate in the other case.

(ii) *Varying methods of stirring* The results obtained with jets delivering radially into the mixing chamber were the same as those obtained with the usual tangential arrangement, although the type of fluid motion imparted to the suspension was quite different in the two cases (Fig 4). Either the violence of fluid motion must have been in both cases sufficient to maintain complete mixing of the suspending fluid,



or have been in both cases inadequate to exactly the same extent, which latter seemed rather an improbable coincidence

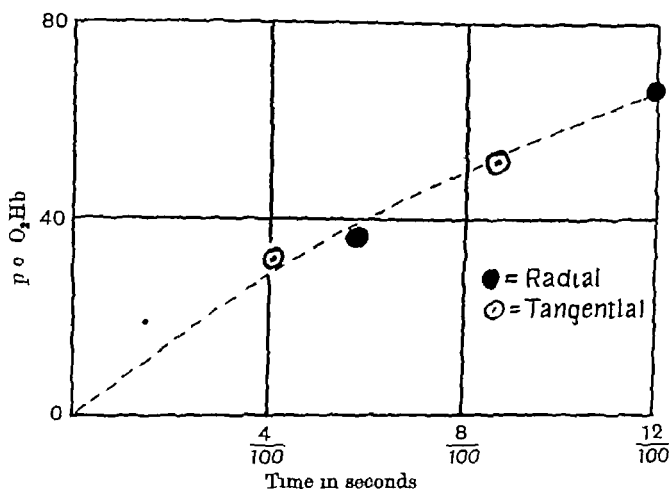


Fig 4.

(iii) *Additional stirring by means of COHb corpuscles* Part of the stirring of the suspension fluid must be due to the irregular motion of the corpuscles. It was therefore thought that the addition of further corpuscles which were prevented from taking part in the  $\text{O}_2$  uptake, might yet, owing to their stirring action, increase the observed velocity

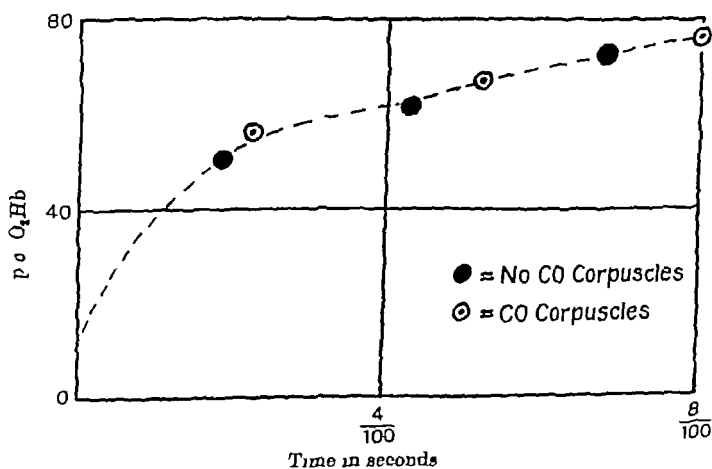


Fig 5

of uptake of  $O_2$  by the corpuscles which do take part in this process. One experiment was first carried out in the usual way. To the aerated 1 p.c. NaCl was then added a quantity of blood, which had been shaken with the minimum quantity of carbon monoxide necessary to saturate the hæmoglobin. The experiment was then repeated as before, with the exception that the COHb auxiliary trough usually employed in the spectroscopic determination of p.c.  $O_2$  Hb was omitted, for its rôle was now fulfilled by the CO-saturated corpuscles. During the short time occupied by the suspension in the observation tube it was not possible, owing to the slow rate of dissociation of COHb, for any appreciable amount of CO to leave the one set of corpuscles and to enter the other set, and thus obstruct the uptake of  $O_2$ , the CO-corpuscles therefore functioned merely as additional stirrers. In spite of such additional stirring, the observed rate of  $O_2$  uptake as shown in Fig. 5 was not affected.

(iv) *Repeated mixing test* The suspension fluid emerging from the mixing chamber, must, according to our previous tests, be completely mixed.<sup>(1, 2)</sup> If, however, concentration gradients develop during the subsequent passage down the observation tube, and thus retard the rate of  $O_2$  uptake, it should be possible to accelerate the latter temporarily by giving the fluid a second mixing after it had passed through a certain length of the observation tube. To achieve this end, a special apparatus with two mixing chambers in series was constructed. The experimental details will be described elsewhere<sup>(8)</sup>. One experiment was performed

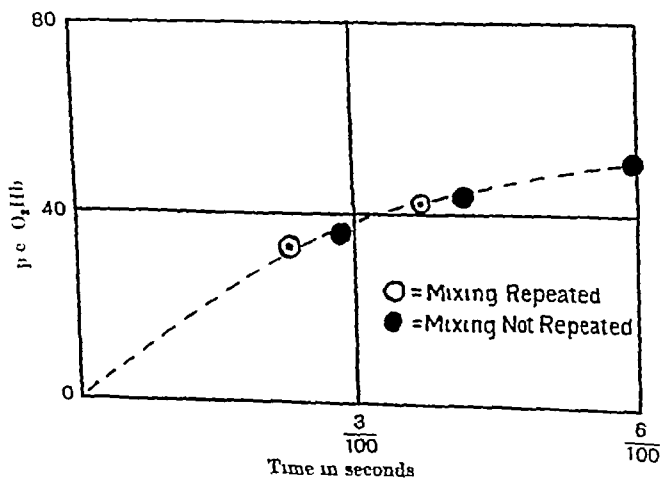


Fig. 6.

in the usual way, and a second one with the rate of flow and interval between the two mixing chambers so adjusted that the p c  $O_2Hb$  in the corpuscles had had time to reach about 30 p c, when the fluid arrived at the second mixing chamber. The results of the two experiments are given in Fig 6, inspection of which fails to show that the second mixing produces any acceleration in the rate of  $O_2$  uptake

The results, then, of these four tests, seem to us to demonstrate quite unequivocally that any gradients of  $(O_2)$  set up in the suspending fluid during the course of the process under examination are of negligible magnitude

### FURTHER EXPERIMENTAL RESULTS

The remaining observations will be presented briefly as their significance has not yet been worked out

(i) *Variation in the initial concentration of dissolved oxygen* A three-fold increase in  $O_2$  increased the rate of  $O_2$  uptake about two-fold (Fig 7)

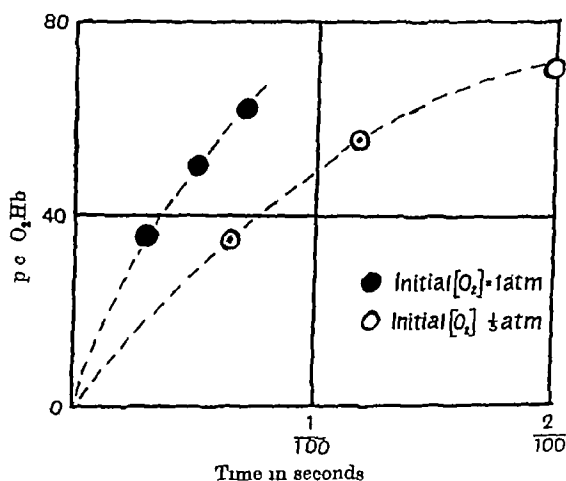


Fig 7

(ii) *Increase of temperature* The reduced corpuscle suspension at  $19^\circ C$  was mixed with

(a) 1 p c NaCl at  $13^\circ C$  Final temperature =  $16^\circ C$

(b) 1 p c NaCl at  $45^\circ C$  Final temperature =  $32^\circ C$

The value of the oxygen concentration in the final mixture was arranged (by shaking with suitable gas mixtures) to be the same in (a) as in (b). No difference in the rate of  $O_2$  uptake at the two temperatures could be detected

(iii) *Increased concentration of NaCl* No difference in rate of  $O_2$  uptake between corpuscle suspensions in 1 and 2 p.c. NaCl respectively was found

(iv) *Comparison of the rate of uptake of  $O_2$  and of CO by corpuscles* Fig. 8 gives the result of a single pair of comparative experiments upon

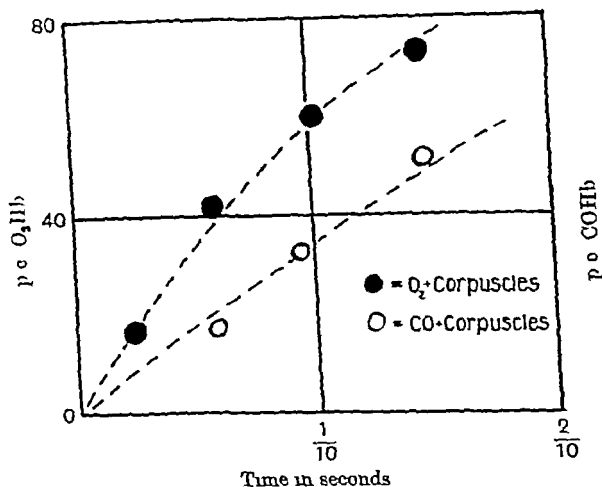


Fig. 8

the rate of uptake, by the same reduced corpuscle suspension of  $O_2$  and CO respectively. The initial concentrations of dissolved gas were, in the two cases, arranged to be the same. It will be noted that the ratio (rate of CO uptake) (the rate of  $O_2$  uptake) is about 1/2, instead of being 1/12, which is the value usually found for this ratio in the case of haemoglobin solutions (6).

These preliminary experiments clearly open up a whole range of further interesting inquiries, but before embarking on these, we feel it essential to settle the two questions already mentioned. (a) Which of the three intermediate processes (*i.e.* diffusion through the corpuscle membrane, diffusion through the outer layers of the corpuscle, chemical reaction inside the corpuscle) is limiting the rate of the final process which we observe? (b) How far can the results obtained on the corpuscle suspensions *in vitro* be applied to the corpuscle *in vivo*? Until the latter question has been answered it would be premature to publish detailed calculations and deductions as to processes occurring in the blood capillary. All we can say at present is that, if the above results hold good for the corpuscle *in vivo* without modification, then our calculations

show (a) that the rate of  $O_2$  distribution within the capillary is so rapid as to have normally no appreciable limiting effect on the rate of  $O_2$  uptake by the blood in the lung, (b) the CO which enters the lung capillaries, during a determination of the diffusion constant of the lung by the Bohr-Krogh method (9, 10) cannot be distributed to the hæmoglobin molecules in the corpuscles fast enough to prevent the back pressure of dissolved CO in the plasma reaching appreciable dimensions. In consequence the diffusion constants calculated by the Bohr-Krogh method would be too low.

### SUMMARY

The rate of uptake of dissolved  $O_2$  by corpuscles has been measured by means of rapid reaction velocity methods. A suspension of reduced corpuscles was mixed with NaCl solution containing dissolved  $O_2$ , and the subsequent entry of  $O_2$  into the corpuscle followed by applying the reversion spectroscope to various points of the observation tube. Four control experiments, performed in different ways, all showed that the suspending fluid during its progress down the observation tube kept itself so well mixed that the observed rate of increase of p.c.  $O_2$ Hb must be a measure of the rapidity of events taking place in the corpuscle alone. A preliminary account of the effect of varying dissolved  $O_2$  concentration, temperature and NaCl concentration is given. The rate of uptake of CO under parallel conditions was found to be about one-half the rate of uptake of  $O_2$ . Further work is in progress to decide how far these results are applicable to the study of the rate of gas distribution inside the living blood capillary.

Our best thanks are due to the Medical Research Council, not only for defraying part of the costs of this research, but also for providing us with a private assistant who has enabled us to reduce considerably the time taken by each experiment.

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# THE REGULATION OF THE ENERGY OUTPUT OF THE HEART

BY E H STARLING AND M. B VISSCHER

(From the Department of Physiology and Biochemistry,  
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## PART I THE INFLUENCE OF MECHANICAL FACTORS

EXPERIMENTS carried out in this Laboratory have shown that in an isolated heart, beating at a constant rhythm and well supplied with blood, the larger the diastolic volume of the heart (within physiological limits) the greater is the energy of its contraction. It is this property which accounts for the marvellous adaptability of the heart, completely separated from the central nervous system, to varying load, to which attention was first called by Cohnheim. So important is this property of the heart for the processes of adaptation and compensation in the healthy and diseased organ that it was called by one of us "the law of the heart"<sup>(1)</sup> and regarded as a special instance of the law connecting energy of the contractile process with the initial length of muscular fibre (Blix<sup>(2)</sup>, A. V. Hill<sup>(3)</sup>). The law as stated above applies only so long as the physiological state of the cardiac muscle can be regarded as unimpaired. As the heart tires it has to dilate continuously in order to maintain its mechanical performance constant, so that the heart *in situ* becomes inefficient, *i.e.* unable to carry on the circulation at the previous output and arterial pressure, so soon as further dilatation is prevented by the heart, during diastole, coming up against the pericardium<sup>(4)</sup>. In such a case freeing the heart from the pericardium will allow it to continue its function and to carry on the circulation as efficiently as before for a certain further period of time. It was shown by Lovatt Evans<sup>(5)</sup> and his fellow-workers that any increase in the work thrown upon the heart in the heart-lung preparation, whether by increasing the arterial resistance or by augmenting the inflow into the heart, caused a corresponding increase in the gaseous metabolism of the organ, whether measured by oxygen intake or by CO<sub>2</sub> output. It was therefore assumed that the increased energy of contraction incident on greater diastolic volume was attended by and due to increased chemical change. Thus



the amount of this latter should also be a function of the initial length of fibre, as has been shown to be the case for voluntary muscle, when the total chemical change is measured by the heat production<sup>(6)</sup> Direct proof of this relation was lacking In Lovatt Evans's experiments no direct measurements were made of the pulmonary arterial pressure or of the coronary flow—both of them highly variable factors when the work of the heart is altered by changing the inflow or the arterial resistance—while alterations in the volume of the heart were inferred by the rough method of observing changes in the level of the blood in the venous reservoir of the heart-lung apparatus It seemed to us that the gaseous metabolism of the heart was of sufficient importance to deserve reinvestigation by improved methods, profiting by the increased knowledge of the mechanical factors involved in the heart beat which have been brought out by subsequent investigation and measuring directly those quantities which had been simply inferred in Evans's experiments

In this present investigation we have confined our attention to the oxygen consumption of the heart, since this serves as a sufficient measure of the total energy set free in the heart during its activity It is a necessary condition of the continuance of the activity of the heart that the process of oxidative recovery during the diastolic period keep pace with the anaerobic breakdown which previously occurs during systole The measurement of the oxygen consumption of the heart is thus equivalent to a measurement of the total heat production during the periods of contraction and recovery

Cardiac muscle offers certain advantages over other forms of muscle for such a study One is the ease of maintaining an adequate circulation through the muscle of a blood well oxygenated, in which the *pH* and the  $\text{CO}_2$  content are maintained constant In the heart-lung preparation the frequency of the beat, provided the *pH* of the blood is constant, depends only on the temperature of the pace-maker, so that by keeping the temperature of the entering blood constant, we can be assured of a regular rhythm throughout the whole of the experiment It is easy, moreover, to maintain the work of the heart constant, or to alter it within very wide limits by changing the inflow or the arterial resistance Finally, the heart may present great variations in diastolic volume and therefore in initial length of fibre within the physiological limits of its activity, accompanied by only minimal alterations in initial tension

Previous work on the gaseous exchanges of the heart have not succeeded in establishing definite relationships between the mechanical

conditions under which the heart is placed and its oxygen consumption. The work of Evans and his collaborators has shown that as the work of the heart is increased its oxygen consumption rises. In different experiments, however, the relation between the work done and the oxygen consumption was extremely variable, and they failed to establish any definite relation between the volume of the heart and its oxygen consumption. Experiments such as those of Rohde(7) in which the mammalian heart is fed by saline solutions are not adapted to solve the questions at issue, though this method may yield results when used with a cold-blooded heart. Observations on the oxygen consumption of the cold-blooded heart have been made by Weizsäcker(8) and by Lüscher(9), and we shall show later that the data obtained by Lüscher, when recalculated, tend to confirm the conclusions arrived at by us as the result of experiments on the dog's heart.

*Methods.* A new and simple method for the study of the oxygen consumption of the heart-lung has been devised. In principle it consists of a measurement of the decrease in the volume of air in a closed system circulating air to and from the lungs, removing the  $\text{CO}_2$  continuously while special precautions are taken to prevent variations in the volume of residual air in the lungs themselves after each deflation. The latter is achieved by the simple expedient of allowing the lungs to deflate themselves by their own elastic tension. Several other methods of achieving this end were tried, but none of them was found satisfactory each introducing large errors. There are several possible errors in the method of allowing the lungs to deflate themselves. The first is that the elastic tension of the lung itself may change over a period of time. This has not been made the subject of a special study in this work because it has been possible to cancel out the possible effects of change in volume by repeating identical observations at various steps in almost all experiments but if very long periods of observations on one preparation were to be conducted with the method here described it would become necessary to allow for the changes in the elastic tension of the lungs. Lung oedema would be a source of great error in our measurements if it were not so easy to detect the beginning of it upon our records. In Fig 1 is shown a portion of a record of an experiment in which oedema of the lungs occurred. Just after the point A there can be seen a change in the slope of the oxygen consumption record, and from that point the slope becomes more precipitous. We have always found lung oedema to have a rapid onset like that illustrated. The detection of such a change is never in doubt, and when it occurs an experiment must be terminated immediately. The beating of the heart freed from the pericardium against the lungs tends to make the spirometer record uneven and difficult to measure, and in such cases we have found it necessary to fix the heart in a cardiometer in order to obtain consistent results. A further precaution we have found essential is to ensure against any portion of the lungs being collapsed at the time of beginning an experiment. It is perhaps needless to say that the lungs must not be handled, or even touched, during the course of a measurement. It is apparent that very many errors must be guarded against if this method is to be found sensitive and satisfactory, and it may be well to mention that we spent four months eliminating sources of error before any useful data were obtained.

In detail, the respiration system employed is as follows: a single cylinder pump (Fig 2, B) of variable stroke volume (Palmer's "ideal" respiration pump) was used, the

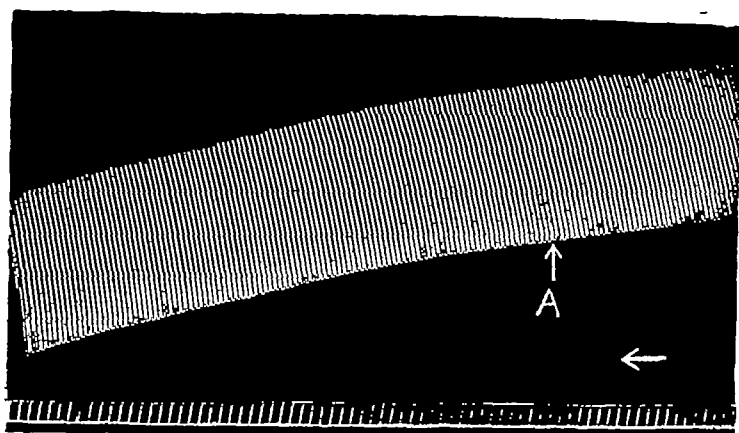


Fig 1 Showing a portion of the spirometer record of an experiment in which oedema of the lungs occurred. The paper was moving from right to left, and the time signals mark each 10 secs. We always measure from the lowest points on the spirometer tracing, as they are much more regular than the highest points. Just after point *A* the slope loses its previous steady character and from that point on becomes steeper and steeper, corresponding with the development of the lung oedema.

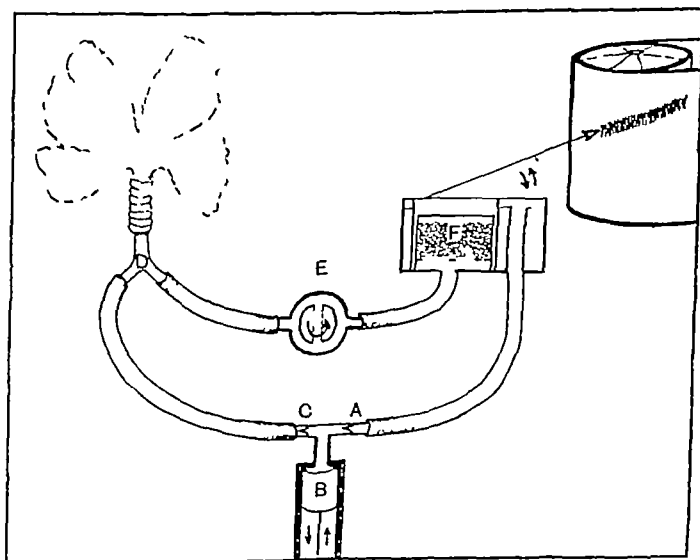


Fig 2 Details of the respiration system employed, described in the text

cylinder, piston and valves of which were very accurately ground to prevent any possibility of leakage at the pressures which come into play in filling the lungs. Air comes to the pump through the valve *A* from the spirometer *F* of 300 c.c. capacity, and is expelled through valve *C* into the lungs, through the forked tracheal cannula *D*. While the pump is forcing air into the lungs the valve *E* is closed. As soon as the inflation is complete *E* is opened and the lungs deflate themselves. They force air into *F*, which is partially filled with soda lime, as in the Krogh apparatus, to absorb the  $\text{CO}_2$  given up in the lungs. The spirometer float has a recording lever which writes on a moving paper, as also do a piston recorder connected with the cardiometer, and a manometer float. The system used is such that 1 mm. fall of the writing lever corresponds to a decrease of approximately 2.5 c.c. in the volume of the system. The change in volume during a single observation period was never less than 50 c.c. Such a volume can be measured with an error of less than 5 p.c. since it involves making only two measurements each accurate to  $\frac{1}{2}$  mm. Repeated measurements always fell within that limit, and usually within  $\frac{1}{4}$  mm. The latter gives a total error of  $2\frac{1}{2}$  p.c. on a measurement of 50 c.c. A rather troublesome complication in measuring changes of volume resulted from the use of a lever writing on the arc of a rather small circle: at different positions of the lever a given fall corresponds to different volume changes since it is the arc and not the perpendicular that is directly proportional to the volume. A correction must be made for the difference. The amount of the correction varies from 0 to 10 p.c. of the total value, the exact amount depending upon the angle the lever makes with the horizontal. The smaller the angle the less the correction.

The heart-lung preparation was made in the usual way (Knowlton and Starling(10)) except that it was converted into a closed system to prevent loss of gases from the blood. This was done by using a collapsible rubber sleeve, immersed in a warm water bath, as the venous reservoir. (A similar system has been employed by Daly(11) for another purpose.) A further change in the ordinary technique of the heart-lung preparation was the insertion of a Stolnikow stromuhr in the pulmonary artery. In order to avoid clotting in the stromuhr this was done after the heart-lung circuit had been established. The Stolnikow stromuhr has been described, in the form used by Anrep(12). In order to measure the volume of the heart a Henderson cardiometer has been employed, fitted with a special rubber sleeve of very thin material, which lay snugly against the heart and made an absolutely air-tight seal around the ventricles without compressing the atrioventricular ring. The volume change was recorded with Palmer's large glass piston recorder. We have measured only the volume of the ventricles, since the atria make up less than 10 p.c. of the weight of the heart, and it seemed reasonable to simplify the problem by neglecting the influence of changes in that small fraction of the total metabolism of the heart upon the final values. Ultimately it may become necessary to reckon with it, but with the present methods, involving a possible error of 5 p.c. in metabolism values, the error involved may probably be neglected.

We have of necessity included in our measurements of oxygen consumption of the heart the oxygen used by the lungs themselves. If the metabolism of the lungs does not alter under the experimental conditions we have used, as the work of Evans and Starling(13) indicates, we are justified in assuming it a constant in a given experiment. The metabolism of the lungs therefore, appears as a constant value added to the real oxygen consumption of the heart. It will in no way interfere with the validity of interpretations or calculations we have made.

In our experiments on the effect of mechanical factors on the energy output we maintained the heart rate constant by keeping the temperature of the heart constant. Previous work has shown that the hydrogen ion concentration in the blood, its  $\text{CO}_2$  content, and its lactic acid content do not change appreciably during the course of an experiment, after

the first quarter hour. This preliminary period we have never employed for oxygen measurements.

The pulmonary arterial pressure was measured by a saline manometer, connected with a branch from the cannula in the pulmonary artery. The aortic pressure was measured by a mercury manometer. In both cases the mean pressure was taken as the basis of the calculations of the work done by the heart.

**Results** The general plan of all our experiments was to determine whether any relation could be established between the consumption of oxygen by the heart and the mechanical conditions of its beat. The resistance to the contraction is determined by the pressures in the pulmonary artery and aorta, so that alterations in these pressures would cause corresponding alterations in the maximum pressures attained in the ventricles during the process of contraction. The work of the heart depends on the output and on the arterial resistance and can be altered therefore by varying either of these two factors. The diastolic volume of the heart will be increased by any increase in either or both of the factors, arterial resistance and venous inflow. The results of our experiments are presented in graphic form, each figure given being a sample of several experiments which yielded corresponding results.

We may deal first with the influence of changes in arterial resistance. In a heart in good condition alterations in the arterial resistance cause

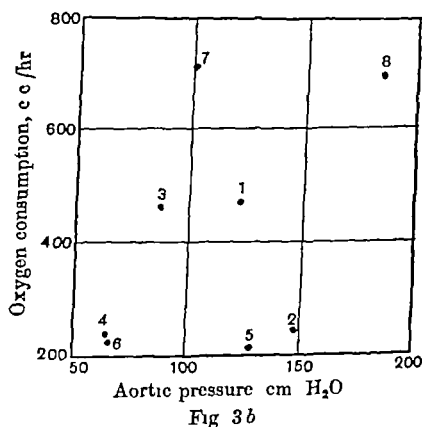
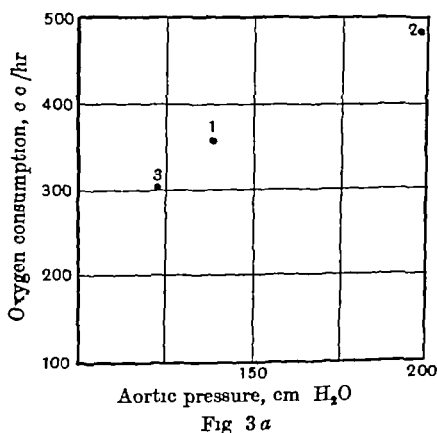


Fig 3 Fig 3a shows data on the oxygen consumption of a heart whose total output was kept constant at 375 c.c. per min., while the aortic pressure was changed. The points are numbered in their time sequence. The oxygen consumption is observed to increase or decrease with the corresponding changes in aortic pressure.

Fig 3b shows data from another heart whose output was changed as well as the aortic pressure. In this experiment there is no proportionality whatsoever visible between aortic pressure and oxygen consumption.

corresponding alterations in the oxygen consumption of the heart. The results of one such experiment are shown in Fig 3a in which changes in the arterial pressure are plotted against changes in the total oxygen consumption of the heart lung preparation. The figures over the dots representing the oxygen consumption indicate the order in which the observations were made. It will be seen that the increase in oxygen consumption is, within the limits of the observations directly proportional to the increment in aortic pressure. Since the isometric part of the intracardiac pressure curve is determined by the arterial resistance it might be thought that here we had a direct relation between the pressure attained in the isometric portion of the curve and the oxygen consumption. Reference to Figs 3b and 4 shows however, that the aortic pressure by itself is not the determining factor. In the experiments, the results of which are given in Fig 3b the venous inflow instead of being maintained constant as in Fig 3a, was altered and now it will be seen that there is no relation at all between the aortic pressure and the oxygen consumption. With increasing inflow there is a corresponding increase in the pulmonary arterial pressure but the relationship between oxygen consumption and arterial resistance is not altered if with varying inflow, as in Fig 4 we plot the oxygen consumption against the *sum* of the pressures in the pulmonary artery and aorta respectively

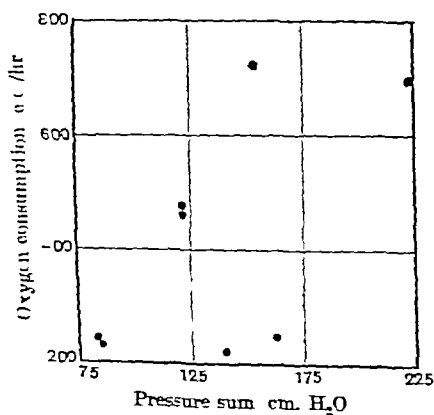


Fig. 4. Data of an experiment typical of many others showing that the pressure sum, i.e. the numerical sum of the pulmonary arterial and the aortic pressures, does not govern the oxygen consumption of the heart.

In the experiment given in Fig 3a, since the inflow into the heart was maintained constant, alterations in the resistance were equivalent

to alterations in the total work of the heart, and if we multiply resistance by output so as to obtain the work of the heart and plot this figure against the oxygen consumption, as has been done in Fig 5 a, we find a definite relation between oxygen consumption and the work. We may see from this and other experiments that as the total work of the heart increases there is a corresponding increase in oxygen consumption. The oxygen usage seems to increase more slowly as the work rises to a maximum, so that it would seem that the efficiency of the heart under these

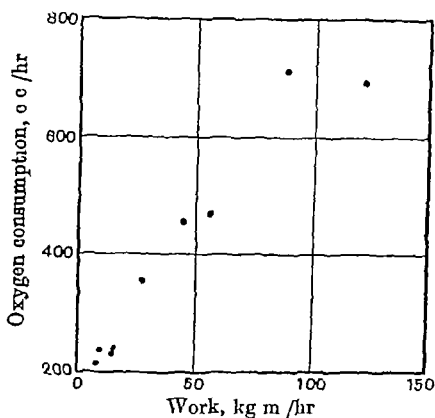


Fig 5 a

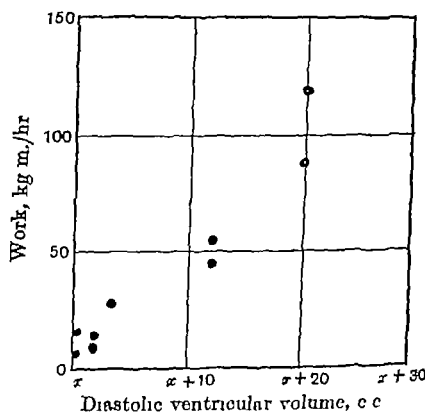


Fig 5

Fig 5 Figs 5 a and 5 b present data from an experiment in which the work of the heart was varied by numerous changes in output and pressure. The outputs ranged from 200-1200 cc per min. and the pressures from 80-120 mm Hg.

Fig 5 a shows the work done plotted against the oxygen consumed. In calculating the work of the heart the velocity factor was neglected. The direct correspondence between the two is very definite in this experiment.

Fig 5 b shows data from the same experiment, showing work plotted against the diastolic volume of the ventricles. The volumes are expressed as  $x$  + known values. The  $x$  represents the lowest value of the volume during the experiment, which is impossible to measure when a cardiometer is used to record heart volume. The cardiometer enables one to measure only an increase over this minimum value. The figure shows a direct correspondence between work done and ventricular volume.

conditions is higher at heavy than at light loads. A similar conclusion was arrived at by Evans. In the experiment given in Fig 5 a the volume of the heart was recorded throughout the experiment, and in Fig 5 b the alterations in diastolic ventricular volume are plotted against the work done by the heart in kilogram metres per hour. It will be seen that there was a steady rise in the diastolic volume as the work thrown upon the heart increased, whether this increased work was due to altera-

tions in the arterial resistance or in the cardiac output (The absolute ventricular volume was not measured. The volume  $x$  indicates the smallest diastolic volume obtained throughout the experiment, and the increments of this volume are plotted along the abscissa against the total external work of the ventricles as obtained by multiplying the total output into the mean pulmonary and arterial pressures respectively.) In Fig. 6 the

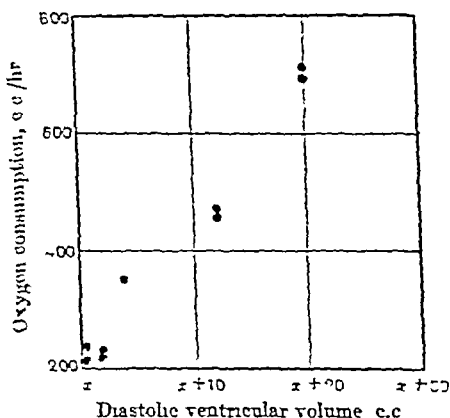


Fig. 6 Shows the oxygen consumption of a heart plotted against its diastolic ventricular volume. This figure is plotted from data from the same experiment from which Figs. 5 *a* and 5 *b* are taken. In the ventricular volumes  $x$  = the lowest volume obtained during the experiment. Many variations of pressure and output were employed. The changes in oxygen consumption are seen to vary directly with the changes in diastolic volume at all stages of the experiment.

oxygen consumption of the heart from which Figs. 5 *a* and 5 *b* were taken, is plotted against the diastolic ventricular volume, a direct proportionality is to be observed between the oxygen consumption and the increments in diastolic volume. These experiments thus lead us to the conclusion that the adaptation of the heart to increased load, whether due to raised arterial resistance or increased output, is effected by dilatation of the heart, this dilatation with the resultant increased diastolic length of fibre being responsible for an increase in the total energy set free at each contraction of the heart muscle.

Our study of the volume and the oxygen consumption of the heart under these varying conditions thus tends to confirm conclusions already arrived at by Anrep as a result of experiments on the cold-blooded as well as on the mammalian heart, namely, that the diastolic volume of the heart depends, not on arterial resistance or on venous inflow alone, but in every case on the product of these two factors, namely, the external work done by the heart at each contraction.



These results, however, apply only to a certain proportion of our experiments. In others, such as that of which the results are given in Figs 7 *a* and 7 *b*, the relation between the oxygen consumption and

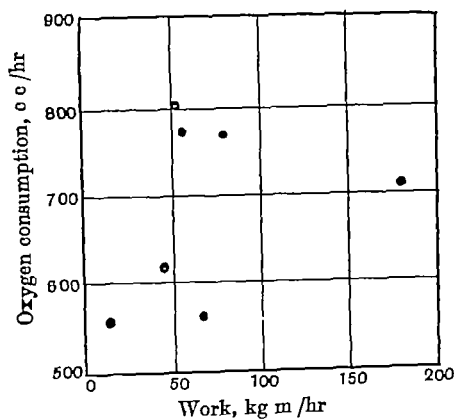
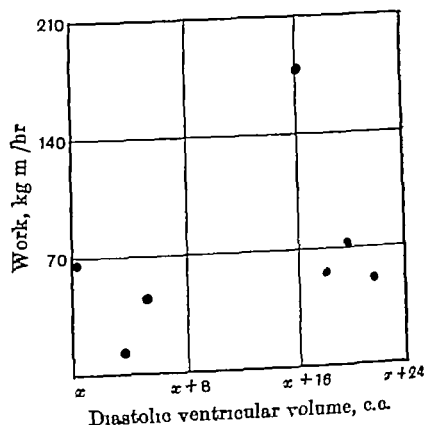
Fig 7 *a*Fig 7 *b*

Fig 7 Fig 7 *a* shows data from an experiment in which the pressure and output of the heart were varied widely, the pressure from 80–160 mm Hg, and the output from 200–1000 c.c. per min. In this heart, which was not in good functional condition (i.e. it did not recover to the same volume when it was given the same load after a period of high work), there is nothing more than a random relationship between work done and oxygen consumed.

Fig 7 *b* shows the relation between the diastolic ventricular volume and the work done. The random relationship is to be contrasted with the proportionality in Fig 5 *b*

work done by the heart or between the work of the heart and the diastolic volume, seems to be entirely random. In order to obtain the results given in Figs 3–6 it is essential that the heart be in good condition and remain in good condition over the period of the observations. The test for “good condition” is that the diastolic volume of the heart remain at a steady level so long as the mechanical conditions of inflow and arterial resistance are unaltered, and that, after changes in volume have been brought about by increase in either of these two factors, the diastolic volume return to its previous level on restoring the previous mechanical conditions. In no case does the heart in a heart-lung preparation remain throughout the experiment in this good physiological state, though in many cases during the first one to one and a half hours it deviates but little from this ideal condition. Generally, however, from the very beginning of the experiment the heart, under even a moderate load, shows a steady slow dilatation although the load is maintained constant,

and the rate at which this dilatation occurs is much increased by further increase in the strain to which the heart is subjected. These are the conditions under which the random relationships between work, oxygen consumption and diastolic volume, such as those shown in Figs 7 *a* and 7 *b*, are obtained. One relationship, however, remains unaltered during this process of deterioration of the physiological condition of the heart muscle, namely, the relation between diastolic volume and oxygen consumption. In the experiment of which the results are given in Fig 8 the work done by the heart was maintained constant for a period of four hours. During this time the heart dilated continuously, the dilatation affecting both diastolic and systolic volumes so that the output at each beat and the total work remained constant. The oxygen consumption also increased steadily, so that, when the oxygen consumption is plotted against the diastolic ventricular volume, we find an approximately

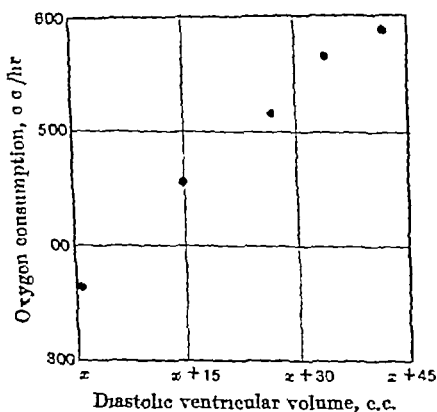


Fig 8

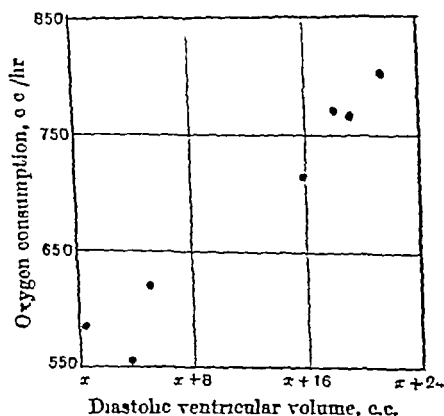


Fig 9

Fig 8 In the experiment here shown the arterial pressure was held constant at 100 mm Hg and the output at 450 c.c. per min. over a period of 4 hours. The figure shows that there was an increase in oxygen consumption concomitant with and proportional to the degree of dilatation of the heart during the experimental period.

Fig 9 Showing the direct proportionality between increments in diastolic ventricular volume and in oxygen consumption in the same experiment from which Figs 7 *a* and 7 *b* are taken. The correspondence between heart volume and energy liberated, even when the work done bears no constant relation to either, is very striking.

linear relation between the oxygen consumption and the increment in diastolic volume. And the same relation is found when the work of the heart is varied in the course of the experiment. In Fig 9 the diastolic volume in the experiment of which the results are given in Figs 7 *a* and

7 *b*, is plotted against the oxygen consumption, and here we find that the random relationship has disappeared and that increase in oxygen consumption goes *pari passu* with increase in diastolic ventricular volume. And in all our experiments similar relations are to be observed (Fig 10)

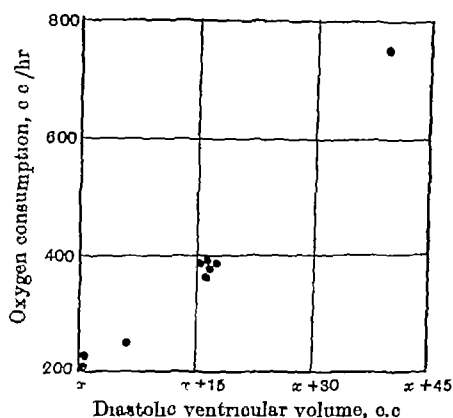


Fig 10 Showing data from another experiment similar to that in Fig 9. Wide variations of arterial pressure and cardiac output were used. The direct proportion between the changes in diastolic volume and those in oxygen consumption is again to be noted.

Provided that the other conditions of the heart muscle, *e.g.* temperature, composition and pH of circulating blood be maintained constant, the oxygen consumption by the heart increases and diminishes with the diastolic volume of the heart. Throughout a long experiment it is possible to keep the diastolic volume constant by gradually reducing the load, and it seems immaterial for the purpose whether this reduction be effected by diminishing the inflow or by diminishing the arterial resistance, provided the arterial resistance is sufficient to maintain an adequate flow of blood through the coronary arteries. Under these conditions it is found that the oxygen consumption of the heart remains at a constant level.

Thus, among the mechanical factors of the heart beat which we have studied, the diastolic volume, *i.e.* the initial length of the cardiac muscular fibres, seems to be the determining factor for the oxygen consumption of the heart, *i.e.* for the total energy set free at each contraction. It is interesting that a similar conclusion may be deduced from the experiments of Lüscher on the frog's heart, the only ones recorded in the literature in which an accurate measurement of ventricular volume has been made simultaneously with a measurement of oxygen consumption. Lüscher believed on *a priori* grounds that the

oxygen consumption of the heart should be directly proportional to the pressure and the ventricular volume. He therefore sought to find if the ratio  $\frac{O_2 \text{ used}}{\text{pressure} \times \text{volume}}$  were a constant. He found as much as 30 p c variation in the value of the ratio and considered this a fair agreement in spite of the fact that he was able to reproduce quantitative results with an accuracy of 10 p c.

Apparently it did not occur to him to test the relationship between simply the ventricular volume and the oxygen consumed. For if one takes his published data (Exps 1, 2 and 3 in Table VII, p 120) and determines the value of the ratio  $\frac{O_2 \text{ used}}{\text{ventricular volume}}$  one finds it to be constant to within 10 p c for a given heart. These values are shown in Table I.

TABLE I.

Table showing the constancy of the ratio  $\frac{O_2 \text{ used}}{\text{ventricular volume}}$  in Lüscher's experiments on the frog's heart, and comparing it with the ratio  $\frac{O_2 \text{ used}}{\text{pressure} \times \text{volume}}$ , which he considered a satisfactory constant. Each experiment was upon a separate heart, and he gives only two determinations on each heart. The second ratio is seen to be nearly constant for each heart.

Exp no	$\frac{O_2 \text{ used}}{\text{pressure} \times \text{volume}}$	$\frac{O_2 \text{ used}}{\text{volume}}$
1	7.4	5.9
	9.8	5.4
2	7.5	7.3
	10.0	7.1
3	8.8	7.3
	10.7	7.0

The facts here presented enable us to give greater precision to the general relationships formulated as the law of the heart, namely, that within physiological limits the larger the volume of the heart the greater are the energy of its contraction and the amount of chemical change at each contraction. We can now state that under all conditions of load, output and fatigue the total energy liberated at each beat is determined by the diastolic volume of the heart and therefore by the muscle-fibre length at the beginning of contraction. This inter-relation between physical dimensions and the amount of chemical change taking place is the explanation of the adaptability of the isolated heart with respect to its load. Under physiological conditions, i.e. so long as the functional capacity of the heart muscle remains unchanged, the mechanical energy of the heart's contraction, as measured by the work it can do, will increase or diminish with the total chemical energy liberated. Thus under

these conditions we may say that the work done by the isolated heart at each beat is determined by its diastolic volume. As the heart tires or as its physiological capacity diminishes in the course of a long experiment, especially when it is given a heavy load, the heart has to dilate more and more in order to carry out the work. This dilatation, as we have seen, is associated with an ever-increasing oxygen consumption, so that the relation of the mechanical work done by the heart in its contraction to the total energy liberated becomes continually smaller. Fatigue of the heart muscle or diminished physiological capacity is therefore determined, not by a diminution in the total energy liberated, but by a diminution in the mechanical efficiency of the muscle regarded as a machine, *i.e.* in the proportion borne by the mechanical work to the total energy liberated.

On a previous occasion one of us has defined tone of the heart as "synonymous with physiological condition or fitness of the muscle fibre" and its measure as "the energy set free per unit length of muscle fibre at each contraction of the heart" (1). This statement is only correct if we insert the word "mechanical" before "energy set free". Probably a more correct definition of tone would be the mechanical efficiency of the muscle fibre, *i.e.* the relation of the mechanical energy to the total energy liberated. The latter, as we have seen, does not change with fatigue but only that portion of it, which can be utilised for the performance of work.

The conclusions we have formulated only hold good so long as the chemical and temperature conditions of the heart muscle are maintained constant. We have no evidence as yet as to the action of alterations in the pH of the circulating blood, which are known to cause changes in the diastolic volume and the rhythm of the heart, but we have definite evidence that adrenaline increases the energy output of the heart at a given fibre length. This it does proportionately at all fibre lengths, so that the relation of energy liberated to initial length of fibre still persists, the only change being that the oxygen consumption at any initial fibre length is increased as compared to the heart without adrenaline.

Till now we have given no experimental evidence that the systolic volume of the heart is not a factor in determining energy liberation. One can, by suitable manipulations of inflow and arterial pressure, alter the diastolic volume, holding the systolic constant. When one does so, the oxygen consumption is not constant, but varies in proportion to the diastolic volume. On the other hand, one can keep the diastolic volume

constant and change the systolic, when one finds that the oxygen consumption does not change but remains steady with the diastolic volume. Data on this point are presented in Table II

TABLE II

Table showing measurements of the oxygen consumption of the heart and of the volume of its ventricles in systole and in diastole. By suitable manipulation of venous inflow and arterial pressure the diastolic volume was changed without altering the systolic volume, and *vice versa*. It is seen that the oxygen consumption does not vary with systolic volume, but follows the diastolic volume of the ventricles.

Experiment	Oxygen consumption c c/hr	Systolic ventricular volume c.c.	Diastolic ventricular volume c.c.	Cardiac output c c per beat	Aortic pressure cm H <sub>2</sub> O	Pulmonary arterial pressure cm H <sub>2</sub> O
19 m. 1926	240	x + 5	x + 7.6	1.3	150	14
	462	x + 5	x + 14	4.5	88	35
	238	x + 5	x + 7.8	1.4	65	16
22 m. 1926	772	x + 10	x + 15.4	2.7	216	23
	768	x + 4.4	x + 16.0	5.8	108	27

## PART II THE INFLUENCE OF THE HEART RATE

In a study of the influence of the heart rate upon the energy cost of cardiac activity two important questions present themselves. First, is the energy output per beat, at a given diastolic fibre length, constant at all heart rates? And second, does the mechanical efficiency of the heart vary with the heart rate?

No satisfactory answer to either of these questions has been arrived at by previous work. Evans(14) experimented with changes in heart rate produced by altering the temperature of the heart, and found the oxygen consumption per beat increased at higher temperatures, at which the heart rate is increased. It is impossible to interpret that result, however, because the heart dilates at higher temperatures and hence one should expect a greater oxygen consumption on the basis of the data presented in Part I. Moreover, the effect of temperature upon the speed of the chemical processes in muscle contraction is not sufficiently well understood to permit one to compare beats at two temperatures under the assumption that they should be alike except for the rate. In fact, the work of Hartree and Hill(15) upon heat production in striated muscle makes such a comparison appear to be invalid. Evans recognized the complexity of the problem and did not attempt to draw a general inference regarding the relation between rate and the energy output per beat from his findings.

We have tried to solve the problem of the relation between heart rate and oxygen consumption per beat by changing the heart rate at a constant temperature, and by taking into account diastolic volume of the ventricles

*Methods* We have experimented with a number of methods of altering the heart rate. We have attempted to "drive" the heart by faradic stimulation of the sino auricular node at a rate faster than the normal rhythm. That method was found unsatisfactory, for most hearts are unwilling to "follow" at a pace appreciably higher than their own. One frequently obtains partial contractions at each stimulus, when by comparing the blood pressure with the myographic record of auricular contraction one finds that only half or a third of the beats are effective in expelling any blood from the ventricles. There is not actually a heart block since inspection of the ventricles shows that they are contracting at each stimulus, but only partially. It is highly improbable that such weak contractions would have the same energy output as a normal one, and consequently we have been forced to adopt other methods. Pilocarpine was tried, and although it reduces the heart rate satisfactorily, it has a stimulating action upon the bronchioles, contracting them and hindering deflation of the lungs, thus making metabolism study by the method we employed impossible. The method of altering heart rate which we have found satisfactory is that of vagal slowing. It has not been possible in the heart-lung preparation to stimulate the vagus nerves electrically for any length of time after the preparation is made. Consequently we kept the brain of the animal alive by perfusing it by means of a second heart-lung preparation. As Anrep and Starling(16) and Anrep and Segall(17) have shown, it is possible to slow a heart by increasing the blood pressure in the brain. By means of the separate heart-lung we were able to vary that pressure, and consequently the heart rate, at will. There is no connection between the circulations in the brain and the heart-lung of the same animal, consequently the oxygen consumption of the brain was not included in that of the heart-lung. The heart rate was recorded by myographic registration of the auricular contraction upon a rapidly moving drum. In determining the rate every beat in a period was counted. The oxygen consumption of the heart-lung preparation was measured as described in Part I. The heart-lung preparation was made in the same manner as before, except that the Stolnikow stromuhr was placed in the aortic circuit instead of in the pulmonary artery. In that way we were unable to measure the coronary blood flow. That inability appeared to be of small consequence for this study. So far as the first question we have tried to settle is concerned, the cardiac output is of no consequence, for the second question the possible coronary flow changes are of some importance, for Anrep and Segall, in work as yet unpublished, have shown that the coronaries are constricted under vagus stimulation and hence the coronary flow is decreased. That effect is not large as compared with the total volume of blood our hearts put out, which was over 700 c.c. per minute, exclusive of the coronary flow. We kept the arterial pressure constant and hence the most important regulator of coronary flow was held steady. The possible effects of the changes in coronary flow will be discussed under the results.

*Results* When the diastolic heart volume is held constant by adjusting the output, and the heart rate altered by central vagus stimulation of the innervated heart-lung, the total oxygen consumption per unit time is slightly less than at the higher heart rate. The relevant data from the experiments performed are given in Table III

TABLE III.

Table showing the oxygen consumption of the heart per beat at low and high heart rates, at a constant diastolic volume. The low rates were produced by central vagus stimulation of an innervated heart. The oxygen consumption per beat is higher at low heart rates than at high.

Experiment	O <sub>2</sub> consumed per hour c.c.	Heart rate beats per minute	O <sub>2</sub> consumed per beat per minute per hour c.c.
28 v 1926	320	107	3.00
	335	125	2.63
	280	92	3.00
3 v. 1926	333	103	3.23
	356	128	2.78

The concordance of the data in these experiments in which we have eliminated any source of confusion from heart volume changes makes it very probable that in reality a heart puts out less energy per contraction at the same initial fibre length when contracting frequently than when contracting less frequently.

In support of this belief we may say that experiments upon changes in rate by temperature, by driving, and by pilocarpine, all yield the same result. We have not placed complete reliance upon any of these other methods, for reasons outlined above, but when one obviates the difficulties in each, as we have been able to do somewhat satisfactorily, the end result always shows the same fact, namely, that the oxygen consumed per beat is higher, the slower the heart rate. We feel that the vagal stimulation experiments are the only entirely satisfactory ones, and we are, consequently, merely mentioning the other results as confirmatory evidence.

With regard to the efficiency of doing work at various heart rates we have found, as Table IV shows, that the heart uses somewhat less oxygen to do a given amount of work when the rate is slow than when it is rapid.

TABLE IV

Table showing the influence of rate upon the efficiency of the heart under a constant load. The heart volume increased as the heart rate decreased in consequence of the fact that the heart had more work to do per beat. In each experiment the load put upon the heart was the same at high and low rates. The aortic pressure was 120 mm. Hg, and the aortic output about 700 c.c. per min. in each case.

Experiment	O <sub>2</sub> consumed c.c./hr	Heart rate beats/min.	Diastolic ventricular volume c.c.	Remarks
28 v 1926	325	151	$x$	
	275	92	$x-9$	Vagal stimulation
	490	168	$x+3$	
	320	107	$x+9.5$	Vagal stimulation
3 v. 1926	414	128	$x$	
	333	103	$x-15$	Vagal stimulation



It was mentioned before that our failure to measure the coronary blood flow in these experiments makes them slightly imperfect, for there is the possibility of a 5 p c decrease in work done during the vagal excitation. Thus the decrease in oxygen consumed per unit of time under vagal inhibition may not represent quite so large an increase in absolute efficiency of the heart. Even allowing for a 5 p c change in the total work, which is ample to cover the possibilities, the hearts would still have been considerably more efficient at the low rates than at the high. Thus it appears that slowing the heart is a means of making it more efficient in carrying the small loads that it has to do when the body is at rest, normally. This is true in spite of the fact that each beat is more expensive at a given fibre length at slow rates than at more rapid ones. It implies that the saving effected by making each contraction do more work, and hence be more efficient mechanically, is more than enough to counterbalance the other effect.

### CONCLUSIONS (Part I)

1 Under all the conditions we have studied, the oxygen consumption of the isolated heart, maintained under constant chemical and temperature conditions, is determined by its diastolic volume, and therefore by the initial length of its muscular fibres. This rule applies whatever the physiological condition of the heart. During the whole of an experiment the oxygen consumption at a given diastolic volume is always the same, whatever the work that the heart is performing at this volume.

2 In a heart functioning well, as at the beginning of an experiment, every increase or decrease in work done by the heart, is accompanied by a proportional increase or decrease in diastolic volume. The diastolic volume is constant for any given amount of work, whatever be the inflow and the arterial resistance. This is in confirmation of Anrep's findings.

3 It follows from (1) and (2) that any increase in the work demanded of the heart is met by a corresponding increase in the oxygen consumption of this organ, consequent on the increased initial length of its muscle fibres.

4 This interrelation between physical dimensions and the amount of chemical change taking place is the explanation of the adaptability of the isolated heart with respect to its load. But as the heart tires, and its functional capacity decreases, its mechanical efficiency is diminished, i.e. although the total energy (as measured by oxygen consumption) liberated at any given initial length of fibre remains unchanged, the fraction of this energy which can be utilised for the per-

formance of work progressively diminishes. To do the same amount of work the heart has therefore to dilate continuously, and the work is maintained constant at an ever-increasing cost in total energy.

5 The oxygen consumption of the heart has no relation to the systolic volume.

6 There is evidence that adrenaline increases the oxygen consumption at a given fibre length, without however altering the general correspondence between changes in diastolic volume and in oxygen consumption.

### CONCLUSIONS (Part II)

1 At the same diastolic length of fibre, a heart uses more oxygen per beat when contracting at a low rate than at a high.

2 Slowing the heart enables it to do a given amount of work per unit time more economically.

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# AUTOMATIC SAMPLING OF ALVEOLAR AIR, PARTICULARLY FOR MEASUREMENT OF THE CIRCULATION WITH ETHYL IODIDE VAPOUR

BY YANDELL HENDERSON

*(From the Physiological Laboratory, Cambridge)*

For measurement of the circulation of the blood by means of ethyl iodide, it is essential to determine not only the rate of absorption of the vapour from the lungs but also the concentration of the vapour in the alveolar air. It is the average concentration throughout the respiratory cycle, inspiration, expiration, and pause that is required. During quiet breathing, the fluctuations in the amount of  $\text{CO}_2$  in alveolar air probably amount to about 5 p.c., the pressure rising from a low point of 38 mm to a high of 40 mm. As ethyl iodide vapour has a coefficient of distribution, or solubility, only a little less than that of  $\text{CO}_2$  in blood, as shown in the slope of the  $\text{CO}_2$  dissociation curve, its fluctuations should be of about the same extent.

Haldane and Priestley(1) considered that the mean alveolar  $\text{CO}_2$  is most nearly estimated by averaging the amounts in samples from a deep expiration at the end of normal inspiration and another deep expiration at the end of normal expiration. Dautrebande(2) supports this opinion on the basis of analyses of the arterial blood. On the other hand, Bock and Field(3) conclude from their observations that the pressure of  $\text{CO}_2$  in the last part of a deep expiration after normal expiration tallies almost exactly with the pressure of  $\text{CO}_2$  in a sample of arterial blood drawn equally during all phases of the breath.

An automatic method of sampling the last of each normal expiration has been devised by Henderson and Haggard(4), and is used as a part of the method of measuring the circulation by means of ethyl iodide. It requires no co-operation or voluntary effort on the part of the subject, other than to breathe through a mouth-piece and valves(5). Most persons over-breathe to some extent for two or three minutes after a clip is placed on the nose and a tube in the mouth, so that at first samples of air somewhat low in  $\text{CO}_2$  are obtained. But increasing

experience shows that this initial excess ventilation soon wears off, and that respiration of normal amount returns within about five minutes in all except especially nervous and excitable persons. Even in such cases, once the subject's attention is diverted from his own breathing, the respiratory centre resumes its normal subconscious control. Indeed this method of obtaining alveolar air is so much less liable to—what we may call—psychological error than that of voluntary deep expiration that at least for all clinical purposes, and most experimental as well, the former may advantageously replace the latter. The fact that the air samples thus automatically obtained agree in their content of  $\text{CO}_2$  with those by the classic technique has been confirmed by Cordero<sup>(6)</sup>, working in the laboratory of Prof Erlanger, and by several other investigators<sup>(7)</sup> whose results will probably be published nearly contemporaneously with this paper. Samples of air taken from the end of normal expiration by means of electrically controlled valves have been used previously also in place of air from deep expiration by Krogh and Lindhard<sup>(8)</sup>, Boothby and Peabody<sup>(9)</sup>, Loeb<sup>(10)</sup>, Trendelenburg<sup>(11)</sup>, Schall<sup>(12)</sup> and by Clark-Kennedy<sup>(13)</sup>.

At first thought such automatic samples from the last portion of the air issuing from the mouth during normal expiration would seem likely to afford the highest concentration of  $\text{CO}_2$ , and the lowest concentration of any inhaled vapour, such as that of ethyl iodide, that would occur during the fluctuations in the composition of the alveolar air during the respiratory cycle. More careful consideration, however, leads to a very different opinion, namely, that the last portion of a normal expiration, at least during quiet breathing, probably affords more nearly the average composition than any other single alveolar sample.

To show how this is true let us suppose that during inspiration and expiration the air flows in and out at a uniform rate, say 250 c c per second. Suppose also that the dead space is one-third of the tidal volume, and that all of this one-third issues first from the mouth in the first third of expiration to be followed by the two-thirds of expiration composed of alveolar air. In successive portions of this two-thirds the pressure of  $\text{CO}_2$  would be continually rising and the amount of ethyl iodide falling.

The essential point to realise is that the air passing out of the mouth at the very end of such an expiration actually left the lungs when expiration was only two-thirds complete. Furthermore, in such schematic breathing as is here suggested, the rise of  $\text{CO}_2$  and fall of ethyl iodide, in the air remaining in the lungs would continue through the first third of the succeeding inspiration, for outside air would only begin to reach

the lungs after the air in the dead space at the end of expiration had been drawn back into them. Thus the automatic sample at the end of normal expiration would consist of air from the lungs at exactly the middle of the period—composed of all of expiration and the first third of inspiration, or two-thirds of the whole cycle—during which the pressure of  $\text{CO}_2$  would continually rise and that of ethyl iodide would correspondingly fall.

In actual operation normal breathing is not so schematic. Yet the real events, as nearly as they can be pictured, seem to contain no element to invalidate the conclusion that the automatic sample of air comes from the lungs at a time very near the middle of the period between the end of one inspiration and the access of an appreciable amount of fresh air to the lungs in the succeeding inspiration. This would make such a sample more nearly the average of the alveolar air than either one obtained by the classic procedure, provided only that the tidal volume is sufficient to sweep out the dead space completely.

In order to put this matter to experimental test a Siebe Gorman mouthpiece with Rosling valves, an arrangement with a negligible dead space, was used. Between the valves and just beyond the expiratory valve small outlet tubes were attached leading to the automatic sampling device, essentially as for the circulation method. The air inspired was drawn from a large spirometer in which the usual amount of ethyl iodide vapour was mixed with the air. On the expiratory side of the mouthpiece beyond the valve a glass tube of 20 mm interior diameter and 35 cm length was attached. At the far end of this tube a second expiratory valve was placed. Until the final expiration this valve was held slightly open.

After the subject (the writer) had breathed through this arrangement of apparatus for 7 or 8 minutes, so as to afford a reliable filling of the sampling tube of the automatic device, he made the deepest possible voluntary expiration. The last portion of this expiration remained in the glass tube between the two expiratory valves, which were now both closed. This air was drawn off directly through the U-tube of iodine pentoxide for analysis. The deep expiration was made in some cases at the end of normal inspiration, in others immediately after normal expiration. At first discordant results were obtained in these determinations (last two columns of table) owing to the tendency of ethyl iodide vapour to condense or dissolve, on the surfaces of rubber tubes and valves. Sampling a deep expiration in the expiratory tube itself, although necessary in this case, involves considerable experimental variations.

On the other hand, in duplicate determinations of the automatic sample close agreement was found. As the concentration of ethyl iodide vapour in the inspired air varied from one experiment to another, it is only the amounts of ethyl iodide, expressed here in hundredths of a cubic centimetre of thuo, within each separate experiment that are to be compared. Typical data are as follows

Experiment No	Automatic sample	Samples from deep expiration	
		End of inspiration	End of expiration
1	78	85	73
2	73	80	—
3	101	105	—
4	84	—	80
5	80	—	77
6	50	57	47
7	95	—	96
8	61	—	58
Average relations	100	108	96

During the first six of these experiments the volume of air breathed was approximately 8.4 litres per minute and the rate 12 per minute, with a tidal volume therefore of 700 c.c. In the last two experiments the breathing was voluntarily quickened to 24 per minute while the volume was allowed to adjust itself without any appreciable degree of over- or under-breathing. The volume per minute rose under these conditions to about 11 litres, and the tidal volume was about 460 c.c. No symptoms of oxygen-want due to inadequate ventilation of part of the lungs, in the manner described by Haldane, Meakins and Priestley (14), occurred in this subject. Such symptoms would indicate conditions in which automatic sampling would not be valid. Their absence certifies that all parts of the lungs are nearly equally ventilated and that the conditions are therefore suitable for the application of automatic sampling.

### Conclusion

Automatic sampling at the end of normal expiration during quiet breathing at rest may be expected on theoretical grounds to afford very nearly the average alveolar air, and this expectation is apparently in accord with the facts. Furthermore, for most practical purposes, automatic sampling of the alveolar air for  $\text{CO}_2$  (on the basis of data here reported only in general) as well as for ethyl iodide vapour has considerable advantages over the classic method of voluntary deep expiration.

My hearty thanks are due to Prof. Barcroft for the hospitality of his laboratory, and to Dr Hartridge and Mr Henry Barcroft for assistance in assembling apparatus.

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## OBSERVATIONS ON THE CONTRACTILITY OF LACTEALS Part I By HOWARD FLOREY

(From the Department of Anatomy, Cornell Medical School, New York )

GASPAR ASELLI in his original observations upon Lacteals<sup>(1)</sup> (1622) noted that they became empty after death

Hewson in his work on the lacteals<sup>(2)</sup> (1769-70) remarked contraction of these vessels in birds Shelden, about whom little is known, also wrote in the latter half of the eighteenth century describing the contractility of the lymphatics of the neck of the dog<sup>(3)</sup> Tiedemann and Gmelin noted that "une ligature provoque le resserrement du vaisseau en aval, son gonflement et sa dilatation en amont," while Colin made the same observation for the horse<sup>(3)</sup> Bowman and Todd found that the thoracic duct contracted slowly to a mechanical stimulus, while Béclard extended this observation to electrical stimuli<sup>(3)</sup> More complete observations were made by Heller<sup>(4)</sup> (1869) and those reported now, made before the present writer was aware of Heller's paper, confirm his statements fully, while somewhat extending them Heller's observations appear to be in danger of being forgotten, for no important English text-book of recent date mentions them, though they are noted in Tigerstedt's *Handbuch*<sup>(5)</sup> Foster's Textbook of 1877 and Schäfer's of 1898 mention them, but in Foster's work they are condemned for lack of proof that the rhythmical contractions are independent of peristalsis

The apparatus used has been described already in another article<sup>(6)</sup>

It is one in which the animal and the microscope is placed in a box constructed so as to maintain the heat of the preparation at about 37° C The magnifications used were 20, 80 and 550 diams

Observations carried out on mammalian mesentery are complicated by the fact that movement is imparted to it by the rhythmical respiratory movement, the peristalsis of the gut and the pulsations of the arteries, especially where they form S-shaped bends

The respiratory movements are satisfactorily damped in these small animals by placing them in a fairly tightly fitting trough, having only a small rectangular opening for the withdrawal of the gut



In the rat, peristalsis causes slight inconvenience, in the guinea-pig it is at times troublesome, but some preparations are obtained in which the gut is quiescent

Preparations also vary in the degree of disturbance from the arterial pulse. No satisfactory way has been found to eliminate this, but it is always possible to select a lacteal for observation where this disturbing factor is negligible

The mesentery of the guinea-pig is extraordinarily transparent and has the great advantage of being, very often, almost entirely free from fat. That of the rat contains much fat, but in nearly all preparations it is possible to find portions of lacteals free from it. The lacteals of the rat present the convenient feature of coursing at a greater distance from the blood vessels than in the guinea-pig. The fat absorption, after a meal, in the rat, rendering the vessels more visible, makes it possible to see the activities of the lacteals in spite of a considerable investment of mesenteric fat

*The guinea-pig* The essential and most striking feature of the activity of the lacteals in this animal is the rhythmical contractility of the vessels by means of which the lymph is actively pumped towards the thoracic duct

The systole of this movement is a diminution of the diameter of the vessel together with a longitudinal shortening, this disturbance being propagated in an orderly manner along the vessel in the direction of the mesenteric glands. This movement appears to be of the nature of a rapid peristalsis moving along the vessel, these disturbances occurring from 8-10 times per minute, though as high as 22 times and as low as twice per minute have been noted. The time intervals between each individual contraction are not absolutely regular, a difference of 2-3 seconds being common. Sometimes a prolonged pause of some 15 seconds occurs. Vessels lying near one another usually have the same rate of rhythm, though portions in the same microscopic field do not necessarily have their systole simultaneously

The peculiarities observed immediately after the preparation is set up will be mentioned later in dealing with the effects of cold and warmth

The duration, at any one point, of this activity, *i.e.* systole and return to pre-systolic condition, is about one to one and a half seconds. The amplitude of contraction is very variable—a diminution to one-third the diastolic diameter being frequently noted. Interspersed between the contractions of large amplitude are smaller ones which sometimes appear as a mere flicker of the wall. It would appear that the amplitude of

contractions and the time intervals between contractions are dependent on the amount of fluid forced in from behind, for with careful focussing it can be seen that a vessel, quiescent in diastole, suffers a slight dilatation as a preliminary to systole, which follows immediately without pause. At the end of systole scarcely any pause occurs, but diastole follows immediately, the vessel in the time interval between individual contractions remaining in the latter state. Occasionally a puckering of the wall can be observed. This is thought to be due to the attachment of fibres in the mesentery to the wall of the vessel.

With the progress of the observation first white then red corpuscles appear in the lymph which before had been perfectly clear except for the presence of an occasional white cell. It is possible with these cells present in the stream to observe the action of the valves which occur at very short intervals in these vessels.

The valves are long folds of endothelial cells, the nuclei of which can be distinguished under the high power. They are, for the most part of the bicuspid type, the cusps in the diastolic condition of the vessel floating freely in the stream. When systole occurs distal to a valve a gush of fluid and corpuscles can be followed through the valve opening a very beautiful eddy forming over the free edges of the cusps by which means they are kept floating in the stream so as to close readily under the appropriate pressure relations. When systole occurs ahead of the valve the cusps can be seen to come together, first at their free edges until a considerable portion of their surface is in contact. They are very efficient when the vessel is active, allowing only a very slight reflux, if any. The wall of the vessel is contractile at the place of insertion of the valves but in many cases in diastole there is a bulging of the wall on the proximal side of the insertion of the valve cusps.

Sometimes the red corpuscles, after a considerable period of observation collect behind the cusp of a valve and remain wedged there.

The presence of the corpuscles also enables one to estimate the speed with which the contents are propelled. In diastole there may be a complete stoppage of the stream, but in systole the corpuscles move so rapidly that they cannot be recognised even under the lowest powers used.

In some preparations lacteals can be observed to be quiescent but the gut peristalsis to be active. In such a case if the lacteal be observed close to its exit from the gut wall it can be seen that the active peristalsis of the gut forces lymph—as indicated by the corpuscular contents—into the first part of the lacteal, but that this lymph is forced only a very

small distance before the corpuscles are deposited on the lower portion of the lacteal wall. That is, the gut peristalsis is capable of pushing the chyle out from the submucosal sinuses or intramural lymphatics but is not sufficient to propel it along the lacteals, if the contractility of the latter is in abeyance.

*The rat* The lacteals of this animal present in all essentials the features described for the guinea-pig. The contractions, however, in most preparations are not so vigorous as in the guinea-pig. The longitudinal shortening is not present but the amplitude of the circular contractions is of the same order as in the guinea-pig. The force of the contraction of these lacteals is sufficient to displace and pull together the fatty tissue lying near them, in fact it was from this peculiar snake-like movement of the fat that the rhythmical contractions were first noticed.

The nuclei of the circular muscle fibres can be well seen in those portions of lacteal free from the mesenteric fat, but the changes in them, if any, undergone in the process of contraction, cannot be made out.

The very finely divided nature of the fat in process of absorption, suspended in the lymph, can be seen from the fact that the lacteals are white by reflected light and a dull salmon colour by transmitted light.

The lacteals of the rat do not exhibit the propagated peristalsis so clearly as those of the guinea-pig. In some preparations it is unmistakably present but in many there seems to be little co-ordination. For example, it is quite common to find an isolated short length of lacteal beating rhythmically while the portions on either side are quiescent, and sometimes, where two lacteals join, one will be beating rhythmically, the wave of contraction being passed on, while the other is completely quiescent or beating with an entirely different rhythm, which may change from time to time. In many experiments the majority of the lacteals are completely quiescent, but in all experiments some, at least, have exhibited good contractions. It is possible that these differences are due to the effects of the operation. Even when the greatest care is exercised, a certain amount of trauma, exposure to air and cooling occurs. The degree of rough handling, which is sufficient to cause stasis in some of the finer blood vessels, results in a preparation in which many of the lacteals are quiescent and only begin to exhibit rhythmical contractions after a considerable time. The influence of cold also can be demonstrated by flooding an active warm preparation with ice cold saline. The movements cease almost at once, to return again in a few

minutes as the preparation becomes warm. This recovery can be hastened by flooding the preparation with warm saline. Since, however, irregularities in activity are met with even in the most successful preparations, it is possible that they may be physiological and normal.

In the rat it is very easy to see, by the use of reflected light, the plexus of lacteals situated on and in the muscular coat of the intestine. Rhythmical contractions of portions of this can always be observed, while on tracing the lacteals centrally the contractions occur to the base of the mesentery.

*The thoracic duct.* For these experiments large guinea-pigs of over 900 gms. weight were employed for the most part. The usual food was mixed with butter. Urethane and ether were used as anæsthetics.

The thoracic duct was exposed in the following way. A longitudinal incision was made over the thoracic portion of the spinal column. The muscle was carefully dissected away from the vertebræ, care being taken to secure immediate hæmostasis. When thoroughly cleared the laminae of three or four of the lower thoracic vertebræ were removed together with the portion of the spinal cord thus exposed, bleeding being controlled by the use of plasticine. Two vertebral bodies were then removed by cutting through the intervertebral discs, and the ribs close to their vertebral joint. In this portion of the operation care was taken not to damage the underlying structures, especially the pleuræ.

Attempts made to remove the vertebræ without the preliminary laminectomy have failed owing to the profuse hæmorrhage, or puncture of the pleura.

When the vertebræ had been removed the animal was placed on a stand in such a position that the wound could be observed through a dissection microscope. The muscle and fat surrounding the aorta and thoracic duct were then removed by means of glass rods till a good exposure of the duct was obtained. The preparation was then packed with warm saline swabs and left some minutes, after which observation was carried out. The duct is not always a single vessel but may be of a plexiform nature. No rhythmical contractions similar to those occurring in the lacteals have been observed in the thoracic duct of such a preparation. Movement synchronous with respiration occurs but this is obviously of a passive nature.

Several observations have been made just after the death of the animal (from hæmorrhage or pleural puncture) but in no case, in which all respiratory movement had ceased, could any spontaneous contractions be observed.

## SUMMARY

(1) Rhythmical, propagated contractions are described in the lacteals of the guinea-pig and the rat

(2) A method of exposing and viewing the thoracic duct from behind is described

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REFLEX EFFECTS OF ACTIVE MUSCULAR CONTRACTION BY SYBIL COOPER (*Research Fellow of St Hilda's College*) AND R S CREED (*Fellow of New College*)

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INTRODUCTION

IN 1893 Sherrington<sup>(9)</sup> recorded the fact that the knee jerk in cats and monkeys may be inhibited by stimulating the proprioceptive afferent nerves from the hamstring muscles of the same side. This may be done in various ways, among others by active contraction of the hamstrings produced by stimulation of the appropriate anterior spinal nerve root. In 1924 Forbes, Campbell, and Williams<sup>(3)</sup> recorded currents of action in the afferent nerve fibres from the cat's tibialis anticus muscle when this muscle contracted. We have been unable to find any other direct experimental evidence that the proprioceptive organs of skeletal muscle respond to active contraction, though many other observers have obtained results by using mechanical stimuli such as passive stretch (*e.g.* the knee jerk and recent work of de Meyer<sup>(2)</sup>, Liddell and Sherrington<sup>(6)</sup>, and Adrian and Zottermann<sup>(1)</sup>). The pain of cramp is presumably due to stimulation of these endings by powerful contraction.

It therefore seemed important to obtain further evidence (*a*) of active contraction being an adequate stimulus for muscle receptors, and (*b*) of any reflex effects produced by such a stream of centripetal impulses. In their second paper Liddell and Sherrington<sup>(7)</sup> showed that the tonic reflex contraction of the knee extensor muscle of the decerebrate cat in response to stretch could be readily inhibited by passive stretch of the hamstring muscles. This is one of the few described reflexes in which adequate stimulation of the receptors in one muscle produces marked effects in other muscles, and therefore we made it the basis of our work.

*Technique* Cats were used throughout these experiments. Under deep anaesthesia the left hind limb was completely immobilised and denervated by section of the crural, sciatic, pudic, obturator, and external cutaneous nerves, and of the following muscles: psoas (*magnus* and *parvus*), tensor fasciæ femoris, glutæi, and the various tendons inserted

in the neighbourhood of the great trochanter. The same procedure was adopted on the right side except that the nerve supply of the quadriceps extensor and hamstring muscles was preserved. All the connective tissue between these two groups of muscles was also carefully divided so far as was compatible with the preservation of their blood supply. The cauda equina was then exposed by removal of the laminae of the 6th and 7th lumbar and 1st sacral vertebrae, the dura was opened, and the ventral root of the 1st sacral nerve was isolated, ligated, and cut as far proximally as possible. The skull was trephined and decerebration at the level of the posterior colliculi performed in the usual way.

After an interval to allow the anaesthesia to pass off, a metal hook in the patellar tendon (previously separated with a flake of bone from its insertion into the tibia) was connected by a cord passing over a light pulley with a hanging weight varying between 900 and 1500 grm in different experiments. The best results were obtained with a load of 1300 grm. The movements of the muscle were recorded by means of a light straw lever attached to this cord just below the pulley and writing on a smoked drum. The movements of the tendon were thus magnified about 75 times, but the inertia of the recording mechanism was such that no conclusions can be drawn as to the absolute extent of the isotonic movement. We have since devised a less crude method of recording.

Rigid fixation of the limb to the table was ensured by steel drills in the upper and lower ends of the right femur and a pelvic clamp. The last mentioned was found to be absolutely essential. In some of the earlier experiments where it was not employed, strong contraction of the hamstrings produced rotation of the pelvis about the fixed femur and apparent shortening of the quadriceps extensor, even after section of the crural nerve.

The preparation, lying on its left side, having been set up in this way, the hamstring muscles were made to contract by stimulating the motor root of the 1st sacral nerve from a coreless Berne induction coil. A short-circuiting key in the secondary circuit was opened for about 2 seconds for each observation. In most cats the only muscles acting on the knee joint supplied by this root are the flexors (Sherrington(8)). But we met with one example of a "prefixed" animal in which satisfactory results were obtained by using the 7th lumbar root, and one example of a "postfixed" animal, in it the quadriceps was also thrown into contraction and the experiment had to be abandoned. Special care was taken to keep the exposed roots warm and moist between each observation.

We wished to determine whether the reflex alterations of extensor tonus were mainly dependent on increased tension or on change of shape of the flexor muscles. For this purpose, in the early experiments the lower attachments of the hamstrings were isolated by a somewhat lengthy process of amputation below the knee. The muscles were then made to contract either (a) under considerable tension from a weight passing over a pulley, or (b) lying loose. In the latter case there could be only slight, if any, stimulation of those sensory end-organs for which the adequate stimulus is stretch. Later, more satisfactory results were obtained by keeping the leg intact and making observations with the knee alternately flexed and fixed in extension.

*Results* When the preparation had been set up as described above, a good knee jerk followed by a shortening reaction demonstrated the integrity of the proprioceptive reflex arc of the quadriceps extensor. If manipulation of the hamstring muscles produced alterations in the decerebrate tonus of the quadriceps, it was also clear that the sensory path from these muscles was intact and that the lumbar cord was in good condition. A slight inhibition of extensor tonus could generally be obtained by kneading the flexor muscles<sup>(9)</sup>, but passive stretch provided a far more effective stimulus. Here the rate of stretch played a part, as might well be expected from Adrian's results<sup>(1)</sup>, a rapid stretch (produced by passive extension of the knee) gave a relaxation three or four times as great as that obtained with a slow stretch.

When the flexors were made to contract by weak faradic stimulation of the motor root, inhibition was generally evident in the extensors. In the early experiments apparent increase of extensor tonus was occasionally observed, but this was always found to be due to imperfect fixation and never occurred when the pelvis was clamped. Omitting the "postfixed" animal from which no records could be obtained, we have found inhibition of extensor tonus as a result of active contraction of the flexors in each of the 12 cats with which we have worked.

It soon became clear that this inhibitory effect was much greater when the flexors were in an initial state of stretch than when they were slack. An observation was considered complete if three records could be taken in rapid succession—two stimulations with the flexors slack (*i.e.* knee flexed), and interposed between them one with the flexors taut (*i.e.* knee extended). If cooling of the cord could be avoided, the resulting record showed comparable relaxations of the extensors with the flexors slack, and a much greater relaxation with the flexors taut. The following are examples of such series of consecutive observations. The



extent of the relaxation is measured on the smoked drum record, and the figures given must be regarded as comparative rather than quantitative

*Load pulling on quadriceps extensor 1300 grm*

Coil distance 24 cm.			
Flexors slack.	Relaxation of extensor	25 mm.	
" taut.	" "	102 "	
" slack.	" "	20 "	

Coil distance 25 cm.			
Flexors taut.	Relaxation of extensor	105 mm.	
" slack.	" "	18 "	
" slack.	" "	10 "	
" taut.	" "	120 "	

Coil distance 26 cm			
Flexors taut.	Relaxation of extensor	21 mm.	
" slack*	" "	0 "	
" taut.	" "	11 "	

\* Visible contraction of flexors

*Load on quadriceps extensor 1000 grm*

Coil distance 25 cm.			
Flexors slack.	Relaxation of extensor	15 mm.	
" taut.	" "	106 "	
" taut.	" "	100 "	
" slack.	" "	32 "	
" slack.	" "	25 "	

Results such as these were obtained in 7 of the 12 animals. In the remaining 5, reflex excitability varied so much from minute to minute, or the extent of the relaxation was so small, that the records with the flexors slack and taut are not comparable.

The records show one or two other points of interest which we are investigating in more detail. At the end of the stimulation, the inter-current relaxation of extensor tonus was often followed by a further relaxation. Some recovery then took place towards the original resting length, but the recovery was generally incomplete. This failure to regain the initial length is probably a "lengthening reaction." Fig. 1 illustrates these features better than the smoked drum tracings. It is an isometric record of the tension in the patellar tendon and was obtained with an improved technique which will be described in a future paper.

*Control experiments* Certain control experiments were undertaken to prove that the effects described were truly reflex and dependent on impulses set up by the active contraction of the hamstring muscles in the proprioceptive endings of these muscles. They were designed to guard against two fallacies which might invalidate our interpretation of

the records. In the first place, the contraction of the flexors might exert some purely mechanical pull on the extensors or their attachments. This

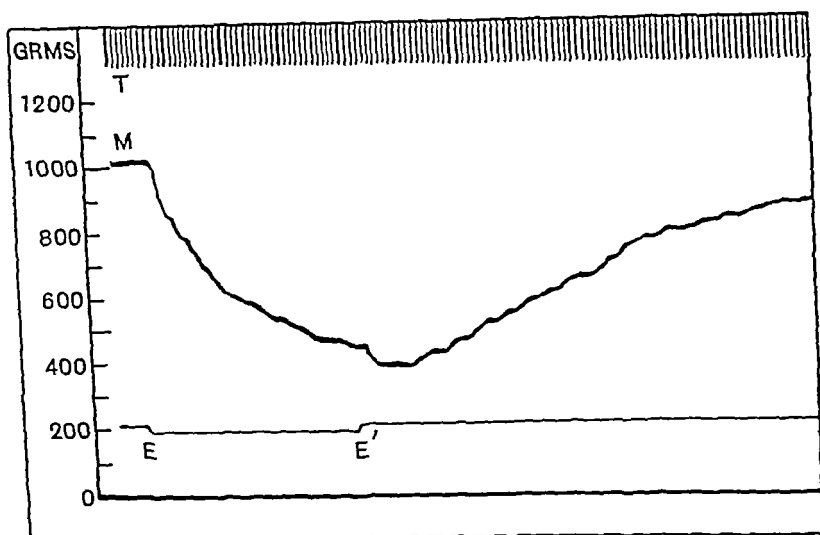


Fig 1 *M* = Photographic record of the tension in the patellar tendon attached to an isometric myograph. *T* = Time in 0.02 sec. *E*, *E'* = Duration of stimulation of motor root to hamstring muscles. Coil distance 24 cm. Tension shown by the scale on the left. Flexors slack. Tension of the quadriceps raised to the initial height by stretch just before the observation.

was unlikely, since the whole limb and pelvis was rigidly fixed, and care was taken that no tight bands of skin or fascia passed between the two sets of muscles. Further, it is difficult to see how contraction of one set of muscles could produce apparent relaxation in a parallel set. But the possibility was finally ruled out when at the end of two experiments we paralysed the quadriceps extensor by cutting the crural nerve. Contraction of the hamstring muscles, produced by much stronger stimulation of the motor root than we had previously been using, then failed to produce the slightest movement of the patellar tendon. On subsequently removing the pelvic clamp such stimulation gave marked apparent contraction of the paralysed quadriceps, undoubtedly due to rotation of the pelvis about the fixed head of the femur.

In the second place, the effects observed might have been due to spread of current from the stimulating electrodes to neighbouring posterior roots. In this case they would still be reflex but would not be caused by impulses arising in the proprioceptive organs of the actively

contracting hamstring muscles. At the end of two other experiments, therefore, the nerve to the hamstrings was cut. In one preparation (which however was already in rather poor condition), this completely abolished the reflex. In the other, the reflex almost, but not quite, disappeared (at coil distance 22 cm. reflex inhibition with flexors taut was reduced from 11 mm. to 1.5 mm., and with flexors slack from 1 mm. to zero), but some muscles in the gluteal region were still observed to be contracting when the root was stimulated. As the nerves to these muscles could not easily be cut, we cut the 7th lumbar posterior root and the cauda equina at the 1st sacral vertebra. No further changes in the tonus of the quadriceps could then be elicited by stimulation of the 1st sacral motor root.

*Discussion.* It seems certain from our experiments that active contraction of the hamstring muscles causes reflex inhibition of tonus in the quadriceps extensor of the same side, and that the inhibition is much more marked if the flexors are prevented from shortening. This points to the conclusion that tension in the muscle is the most important stimulus to the muscle receptor organs concerned, but does not exclude change of shape of muscle fibres as a subsidiary adequate stimulus. Forbes, Campbell, and Williams<sup>(3)</sup> at the end of their paper on afferent nerve impulses from muscular receptors in the cat say, "Clearly our data all point to tension in some form as the source of excitation," but here again it is not clear that change of shape is not also "adequate."

Liddell and Sherrington<sup>(6)</sup>, p. 219) have produced strong evidence that the receptors involved in the stretch reflex are situated in the fleshy part of the muscle and not in the tendon. Probably the same receptors are concerned in the reflex under discussion, but it is impossible to specify their nature until more is known about the various types of sensory endings in muscle and their adequate stimuli.

Our observations are of some interest as evidence of a muscle's power of "recording" its own movements and thereby affecting other muscles. We hope that they may throw some light on the mechanism of "reciprocal innervation of antagonistic muscles." At present it is quite unsettled to what extent this coordination is effected by central or by peripheral means. Graham Brown<sup>(4,5)</sup> in 1914 advanced the view that the stepping reflex is brought about wholly by central action, while Sherrington<sup>(10)</sup>, p. 80 *et seq.* and <sup>(6)</sup>, pp. 236-239) has pointed out that proprioceptive impulses probably provide an important adjuvant stimulus in such rhythmical acts. Our results suggest that impulses coming into the cord from contracting muscles may be partly responsible

for the synchronous relaxation of their antagonists. Thus at the end of the extension phase in a step, the knee flexors are under a slight degree of passive stretch, & *i.e.* they are in the most favourable position to cause inhibition of the extensors when the flexion phase begins. Adrian and Zottermann<sup>(1)</sup> have shown that in a single muscle receptor, there is a short period of complete inactivity following release of the muscle from tension. If this occurs in the flexors at the end of the active contraction in the flexion phase of stepping, the extensors will be released from all inhibitory influence and by possible combinations of a rebound contraction and a stretch reflex the turning point of the step might be effected.

### SUMMARY

1 Active contraction of the hamstring muscles in the cat produced by electrical stimulation of the 1st sacral motor spinal root provides an adequate stimulus for proprioceptive endings in these muscles.

2 Impulses set up in this way inhibit decerebrate tonus in the quadriceps extensor muscles of the same limb by a reflex action.

3 Increase of tension rather than change of shape seems to be the effective stimulus for the elicitation of this reflex.

4 The possible bearing of these experiments on the mechanism of reciprocal innervation and stepping is suggested.

Sir Charles Sherrington suggested this enquiry and we take this opportunity of expressing our indebtedness to him.

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# VASCULAR REACTIONS OF THE SKIN TO INJURY

Part VIII The resistance of the human skin to constant currents, in relation to injury and vascular response<sup>1</sup>

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IN a series of recent papers Ebbecke<sup>(1)</sup> has described a number of interesting observations upon the resistance of the human skin to galvanic currents, and comes to conclusions that, if true, would be of far-reaching importance to the study of vascular responses of the skin to various stimuli

He points out that it has long been recognised that estimates of skin resistance obtained by means of galvanic currents are very much higher in quantity than are the true resistances as measured by alternating currents of high frequency. Using small contacts, the alternating current measures the resistance in terms of a few thousand ohms, using constant currents, values of as much as 500,000 ohms are commonly obtained. The high values shown by the galvanic current Ebbecke probably rightly attributes to polarisation. He finds that these high resistances are broken down by one of two chief procedures, namely, either by the continued passage of the galvanic current, when the latter is employed in sufficient strength ultimately to produce whealing of the skin (local galvanic reaction), or by rubbing the skin. He believes that change in the epidermal cells is responsible for this lowering of resistance, and arrives at this conclusion by a process of elimination. The change is not due to altered vascularity, because it occurs equally well when the circulation to the skin is stopped or in the skin of bodies investigated some hours after death. In the last circumstance Ebbecke believes that the epidermal cells are still living and capable of reacting to stimulation. The sweat glands are excluded mainly on the ground that the local galvanic reaction can be obtained conspicuously upon the skin of the forearm, where sweat glands are scanty, but fails on the hand, where sweat glands are numerous. These two exclusions leave the epidermis for consideration. He considers the

<sup>1</sup> Work undertaken on behalf of the Medical Research Council. Parts I-VII appear in *Heart*, vols. XI-VIII.

<sup>2</sup> Travelling Fellow of the Rockefeller Foundation.

horny layer of the skin and describes observations, which, so he believes, show that when this is particularly dry it may offer much resistance, and that its resistance may fall if the electrode is pressed heavily upon it or if the electrode is wet. In both instances altered resistance is attributed to increased moisture of the horny layer. This change in the resistance of the horny layer introduces an error that may conceal the more significant changes in the cellular layers that lie beneath it, for this reason Ebbecke describes it and his attention then fastens upon the living epidermis. To explain the lowering of resistance to galvanic currents, he supposes that the act of rubbing the skin, or of passing a galvanic current through it, excites the epidermal cells to activity. This activity is regarded as associated with a dispersion of the polarisation charge and with an increased permeability of the cell membranes, thus stimulation of the skin is brought into line with Bernstein's hypothesis, as this is applied to muscle and nerve. Further consideration of his conclusion, that lowered resistance to galvanic currents is brought about in this way, is important from several standpoints. Upon it Ebbecke mainly bases a hypothesis that vascular changes in the skin and whealing, consequent upon the passage of galvanic currents, or in response to mechanical stimulation, results from the release of cellular metabolites, this release being a consequence of increased permeability. One of us, working in conjunction with Grant(2), came independently to a similar conclusion, in respect of the intervention of metabolites in reactions to injury, though from observations of a distinct kind.

When we became aware of Ebbecke's work upon skin resistance, we were inclined to the view that his observations might constitute important evidence in favour of the reaction of the vessels to cellular products, and that they might materially help to establish, as a conclusion, the idea we were postulating. It also seemed to us that Ebbecke's method and conclusions, if sound, might be of great value as a basis in investigating the time-relations of the skin's cellular activity, that it might be possible to find further parallels between the skin's capacity to conduct constant currents and the response of its vessels to cutaneous injuries, and that his work would enable us to measure accurately the period, short or long, over which cellular activity in response to various injuries lasts, and to assess more accurately the intensity of the reactions.

We have been disappointed from all these points of view, and an initial hesitation in accepting Ebbecke's attractive interpretations of his experiments has grown into a conviction that they are unsound. The

following observations are selected to show our grounds for this conviction

*The visible reaction of the skin to a weak galvanic current* In the last paper of the series referred to Ebbecke uses the following method A battery of 4 volts is connected to the body by means of two electrodes The one, an indifferent electrode, consists of a vessel of water, and into this a hand is plunged The other consists of a fine needle, this is laid very carefully so that its point lies on the skin of the forearm When the current is made, and the needle point constitutes the kathode, little or no current is at first registered as passing through the skin In less than 15 seconds the current increases abruptly and reaches a value of 70-100 microamperes, and the subject experiences burning pain at the needle point When the needle is removed, a minute shiny or slightly brown spot is seen on the skin This is soon associated with reddening, and before long the skin rises to form a small wheal, like that produced by the bite of an insect The effect is obtained only if the needle point is kathodal, and Ebbecke shows that the lowering of resistance in this experiment occurs only at the kathode, and he concludes that the essential kathodal action consists of an increased permeability of the epithelial cells

We have repeated these experiments and agree with the results he describes in all their details, but we place a different interpretation upon them If the current is led through the skin in the manner described, and the needle point is watched microscopically<sup>1</sup>, we find that at or about the instant when the passage of the current becomes painful, minute bubbles of gas are seen to form around the needle point and others collect within the horny layer of the skin, lifting and disrupting it In a large number of experiments there has been no exception to this statement, and we have never succeeded in obtaining subsequent whealing of the skin with currents of insufficient intensity to produce this visible electrolysis of the tissues The little shiny or brownish mark on the skin that Ebbecke describes is the result of the disruption of the superficial layers of the skin by these gas bubbles We do not go so far as to deny that the passage of a constant current may not alter the activities of the epidermal cells, with associated changes in their permeability, but it is clear that the cutaneous reaction described by Ebbecke follows a gross injury of the skin, and it will become evident later that the lowering of resistance that occurs is not due to the cause to which he ascribes it, but to an electrolysis associated with mechanical injury of the skin's horny layer

<sup>1</sup> We have used Greenough's binocular microscope and 60 magnifications for the purpose.

*Relation between the galvanic resistance and the visible skin reaction*

**Mechanical stimulation** One hand is placed in a vessel of normal saline in which a piece of silver foil coated by electrolysis with silver chloride is immersed. The second electrode is placed on the other arm, it consists of a glass tube of 4 millimetres internal diameter, filled with gelatine, made up into a firm jelly with normal saline and having embedded in it a similar sheet of silver foil coated with silver chloride. These electrodes show no detectible polarisation phenomena. A current from a 2-, 4- or 6-volt battery is led through this circuit, and the current recorded rarely exceeds 5 microamperes<sup>1</sup>, providing that the small electrode lies on healthy and undisturbed skin. It has been our habit always to moisten the skin so tested with a drop of normal saline a minute before applying the contact. Removal and replacement of the contact has little or no influence upon the amount of current flowing. If the current at first amounts to or exceeds 5 microamperes, it is often found that the apparent resistance tends to become lower after an interval of several minutes, but if, as is usual, the preliminary reading is decidedly less, this tendency for the resistance to fall is usually absent or trivial.

If the contact is removed and the skin vigorously rubbed or firmly stroked with a blunt point, and the contact replaced at once, the current now flowing is found to have risen abruptly, though by varying amounts. The resistance, originally of 100,000 to 1,000,000 ohms, falls to such values as 50,000 and 10,000 ohms, exceptionally even to 5000 ohms.

*Observation 1* Skin of forearm connected to battery of 4 volts. Current readings in microamperes. Five subjects.

Before stroking	After stroking						Remarks
	Immediate	1 min.	2 mins	3 mins.	4 mins.	5 mins.	
0.25	—	25	24	36	30	27	Normal skin. Heavy stroke. Skin unbroken. Local red reaction only
3.0	600	625	635	600	600	600	Normal skin. Heavy stroke, flakes of horny layer torn off. Local red reaction only
0.25	17	22	20	20	21	21	Normal skin. Heavy stroke Very slight whealing
0.25	—	400	400	420	450	450	Normal skin. Heavy stroke. Slight whealing
0.7	—	2.0	2.5	2.5	2.5	—	Case of urticaria factitia. Moderate stroke Full wheal in 2 mins.

<sup>1</sup> Our object in changing the voltage is merely to obtain a definite reading of current with our meter in all cases the meter is sensitive to  $\frac{1}{2}$  microampere and it often happens that with 2 volts no movement of the needle is recorded.



The observation agrees precisely with Ebbecke's findings and we agree with him in stating that the reaction is unchanged when the circulation to the limb is stopped. There is, however, no relation between the fall of resistance and the reaction that follows. A heavy stroke may lower the resistance to 1/200 of what it was originally and result in a local red reaction only. In the case of a susceptible skin, a stroke of moderate firmness may produce a full wheal and the fall of resistance may be inappreciable by comparison. If flakes of the horny layer are carried away by the stroke, the fall of resistance is profound, yet the reaction of this skin may be comparatively slight.

If, instead of rubbing the skin, we employ a fine needle, and prick the skin, similar falls of resistance occur. It occurs with regularity and is immediate, and it will happen with needle pricks of surprising lightness.

*Observation 2* Skin of forearm connected to battery of 2 volts. Current readings in micro-amperes. Four subjects

Before pricking	After pricking				
	Immediate	1 min.	2 mins.	3 mins.	4 mins.
1 25	145	125	80	70	65
4 5	75	95	80	95	85
1	125	100	100	100	110
1	65	50	50	50	45

The prick necessary to break the resistance is far less than sufficient to draw blood from the skin, and often it is unaccompanied by a sensation of pricking or by visible response of the vessels, it is sufficient apparently for the needle point to pass into the horny layer of the skin. Here again the effect is obtained if the circulation to the skin has been stopped previously. If the skin has been lightly pricked in a few places, these places are easily detectable by passing the contact over the skin some while afterwards, the instant such a point is reached, the galvanometer needle moves rapidly from almost zero to a reading of 70-150 micro-amperes.

Exactly similar effects are to be obtained upon skin removed from the body. The skin we have used has been taken from post-mortem subjects, within a few hours of death or in some cases 24 or more hours after death, in the last cases the bodies have lain in a cold chamber. The capacity of such skin to conduct increases, so that the values for the current flow before and after pricking gradually approach each other as time elapses and as decomposition occurs in the skin. The effect of the prick on skin conduction is not dependent upon survival of the cutaneous cells, as is clearly shown in the following way. The skin is

immersed in 40 p.c formaldehyde for 30 minutes or longer, it is taken out, the surplus fluid mopped away, and the skin allowed to dry a little at room temperature until it presents no trace of surface moisture and until drops of saline placed upon it remain in place. Skin so treated behaves as does normal living skin, a prick promptly reducing its resistance very greatly.

*Observation 3 a* Skin from post-mortem subject. Connected to battery of 2 volts. Current readings in microamperes.

		Reading
12 hours after death (2 hours at room temperature)	Before pricking	2
	After pricking	100
	Before stroking	2
	After stroking	22
36 hours after death (6 hours at room temperature, remainder on ice)	Before pricking	50
	After pricking	120
60 hours after death (10 hours at room temperature, remainder on ice) Skin decomposing	Before pricking	75
	After pricking	120

*Observation 3 b* Skin from post-mortem subject 18 hours after death (2 hours at room temperature) immersed for 30 minutes in 40 p.c. formaldehyde. Fluid mopped off surface. Connected to battery of 2 volts.

		Reading
1st point tested	Before pricking	2.5
	After pricking	100
2nd point tested	Before pricking	20
	After pricking	125

Skin retained in small moist chamber for 24 hours the skin is rigidly hardened re examined.

3rd point tested	Before pricking	2
	After pricking	85
4th point tested	Before pricking	1.5
	After pricking	110

*Freezing* If the normal living skin is hard frozen and allowed to thaw, it becomes brightly reddened and soon the skin swells to form a prominent wheal. The reaction of the skin is of the most intensive kind, but at no stage can a material fall if its resistance to constant currents be detected.

*Observation 4 a* Skin of forearm. Frozen at  $-13.3^{\circ}\text{C}$  for 5 seconds. Battery of 2 volts. Readings in microamperes.

	Frozen area		Control area
Before freezing	2.5		
After freezing			2.0
3 minutes	2.0	Slight whealing	
7 minutes	0.7	Wheal distinct	1.5
45 minutes	0.5	Wheal declining, skin red	1.0
			0.5

*Observation 4 b* Skin of forearm. Frozen at  $-18.8^{\circ}\text{C}$  for 10 minutes. Skin hard and deeply frozen.

	Frozen area		Control area
Before freezing	2		2
After freezing			
2 minutes	6		2
4 minutes	7	Wheal starting	3
38 minutes	1	Wheal full	1
24 hours	0.25	Wheal still present and tender	0.75
1 to 7 days	Repeated readings showed no fall of resistance, or difference between frozen and control areas.		
10 days	1200	Horny layer scaling off	4
11 days	800		12
12 days	100		1

*Burning heat* If living skin is burned by placing it in repeated contact with a test-tube of scalding water until widespread redness and slight swelling of the skin is produced, the effects of this reaction of the living cells upon the resistance to galvanic currents is likewise absent or inappreciable, throughout the whole reaction

*Observation 5* Skin of forearm. Burnt with test-tube of scalding water repeatedly applied until a vivid local red reaction, with surrounding arteriolar flare, was obtained. Battery of 4 volts. Readings in microamperes.

	Burnt areas		Control areas	
	1	2	1	2
Before burning	1.0	0.75	3.5	1.0
After burning	2.0	0.5	4.75	0.75

*Ultraviolet light* If the skin is burnt with ultraviolet light so that it reddens within 1 or 2 hours and is red and swollen on the subsequent day, no appreciable change of skin resistance is to be detected throughout this period of time. If the resistance of the skin is followed for a period of many days, then on the day when the superficial layers of the skin, which has become discoloured and wrinkled, begins to scale away, a conspicuous fall of resistance is frequently found to have occurred

*Observation 6* Skin of forearm. Ultraviolet light burn, mercury vapour lamp at 18 inches for 6 minutes. Battery of 6 volts. Readings in microamperes.

Time after exposure	Irradiated areas			Control areas	
6 minutes	2.5	—		1.75	—
35 minutes	—	0.25		—	1.5
81 minutes	5.0	7.0	Redness starting	2.0	5.0
24 hours	0.5	0.75	Bright red and swollen skin	0.25	3.5
96 hours	3.0	2.0	Redness fading no swelling	2.5	3.5
144 hours	150	8	Horny layer wrinkled	12.5	5.5
216 hours	1350	1800	Horny layer scaling removed several hours previously by gentle friction	5.5	5.5

*Blistering* We applied a strong cantharadin plaster to the skin of the arm. On the next day the resistance was taken from the skin covering the blister and from control areas, these blisters were filled with clear fluid and usually stood well above the level of the surrounding skin. In none of these tests was a decreased resistance detected. If, however, the layer of dead skin covering the blister was pricked lightly with a needle point, an immediate and profound fall of resistance invariably resulted.

*Observation 7* Skin of forearm Blistered 24 hours previously Battery of 2 or 4 volts  
Readings in microamperes. Two subjects

Blistered skin		Control skin
9		8
1000	Horny cover of blister removed	11
4		4
210	Horny cover of blister lightly pricked	4

### DISCUSSION

These observations seem clearly to establish that the profound changes in the skin resistance (to galvanic currents) described by Ebbecke are not due to stimulation of the living cells, nor to consequent change in the permeability of their walls. The high resistance found in the uninjured skin resides in the superficial and horny layers of the skin and not in the living cells, and the profound changes of resistance occurring in response to the passage of the galvanic current itself, to vigorous friction, and to superficial needle pricks, are due to breaks in the continuity of this horny layer. It follows that his observations upon skin resistance may not justifiably be used as evidence that various stimuli, mechanical and galvanic, when applied to the skin, produce a reaction increasing the permeability of the epidermal cells with an associated release of metabolites, which proceed to act upon the blood vessels, dilating them and increasing their permeability. However suggestive Ebbecke's observations upon skin resistance may be, they can no longer be regarded as providing support to the view that tissue metabolites regulate the blood flow through, or the permeability of, the minute cutaneous blood vessels, and the method in its present form does not seem to offer any prospect of substantially increasing our knowledge of such regulation.

## CONCLUSIONS

The high resistance displayed by skin to galvanic currents resides in its superficial and horny layer and not in the living cells. Conspicuous changes in this resistance are not due to stimulation of the living cells but to breaches in the horny layer.

## REFERENCES.

- 1 Ebbecke Archiv f. d. ges. Physiol. 190 p 230 1921, 195 p 300 1922 and 199 p 197 1923
- 2 Lewis and Grant. Heart, 11 p 209 1924

# THE ACTION OF THE VAGUS ON THE SPLEEN

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BULGAK's paper<sup>(1)</sup> in 1877 on the innervation of the spleen may be considered to be the first systematic study of this subject. A few years later Roy<sup>(2)</sup> published the results of his plethysmographic studies, in which he obtained a series of remarkable tracings with his "oncometer". These investigations showed that the main nerve supply of the spleen is derived from the splanchnic nerve, stimulation of which causes a powerful contraction of this organ.

Very little work has been done, however, on the action of the vagus on the spleen. As is well known, most of the viscera are innervated by two different nerve systems, namely, the para-sympathetic and the sympathetic, with actions entirely antagonistic to one another but maintaining a balance of tone under any given circumstances. It is surprising therefore that in the case of the spleen considerable work has been done on the action of the sympathetic while that of the para-sympathetic still remains obscure, and it was with the object of throwing more light on this obscurity that the present research was undertaken. During the carrying out of this investigation, which was begun in October, 1925, a paper was published by Skramlik and Duran-Cao<sup>(3)</sup> treating of the same problem and reporting new facts which will be referred to later in this paper.

*Method* All my experiments were made on cats, in which animal, as the late Prof. Langley pointed out to me, the spleen is very rich in contractile tissue. Under urethane and E.C. mixture narcosis the abdomen was opened, a part of the omentum severed, and the spleen, freed from stomach and pancreas, enclosed in a special spleen box made of ebonite with a celluloid cover. In opening the abdomen I did not cut in the nipple line, as is usually done<sup>(4)</sup>, but made one incision in the middle line, a second cut at right angles being made in the left side of the abdomen after ligaturing the muscular vessels. In this way the compression or twisting of the splenic vessels was eliminated to a great extent. The blood-pressure in the carotid artery was recorded simultaneously with the splenic contractions.

*Results* (1) *Stimulation of the peripheral end of the vagus* As early as 1868 Oehl(5) stated that the stimulation of the peripheral end of the vagus provokes the contraction of the spleen. He arrived at this result by direct inspection of the change in volume and colour of the organ caused by the stimulation, and it was confirmed by Roy(2) by the plethysmographic method. Being convinced that the spleen communicates with the general circulation by a very narrow path and consequently is very little affected by a change in the systemic blood-pressure, Roy ascribed this contraction to the direct action of the nerve upon the spleen. Bulgak(1), on the other hand, observed no contraction and suggested that Oehl's results were very likely due to the change in respiration caused by the section of both vagi. Later Schafer and Moore(6), using the plethysmographic method, came to the conclusion that the stimulation of the peripheral end of the vagus had no effect upon the spleen. According to them Roy's results might have been caused secondarily by contractions of the œsophagus and stomach which they occasionally observed. The question, therefore, still remains undecided.

In all my experiments stimulation of the peripheral end of the vagus in the neck always caused a more or less obvious diminution in the spleen volume and on some occasions was very marked (Fig 1). This diminution, however, is only brief and is followed by a series of intensified wave-movements, and in a great many cases, after the initial contraction is over, the volume is slightly increased. After several minutes, however, the organ usually returns to its original volume.

So far my results agree with those of Oehl and Roy, but doubt is thrown on their interpretation by closer examination. In the first place, the diminution in spleen volume usually occurs a few seconds after the beginning of the fall in blood-pressure. In the second place, the change in volume is, generally speaking though not always, proportional to the change in blood-pressure. If the fall of blood-pressure is large, the diminution

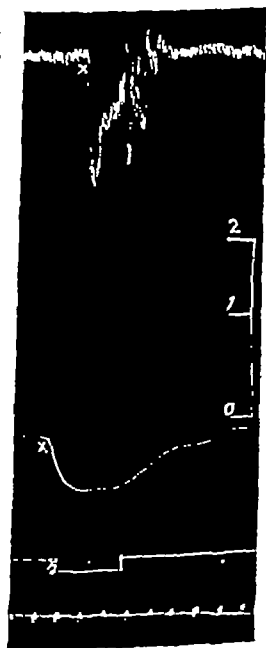


Fig 1 Stimulation of the peripheral end of the vagus. Upper curve blood pressure. Middle curve spleen volume. Lower curve base line of blood pressure. Time 10 secs.

in volume is also large. Moreover, in some cases in which the stimulation was continued for a longer period, the volume varied as the blood-pressure rose and fell (Fig. 2). This fact seems to show that the volume

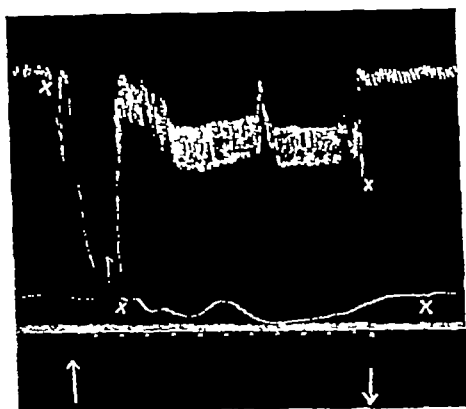


Fig. 2 Long lasting stimulation of the peripheral end of the vagus. Parallelism between blood pressure and spleen volume. Upper curve blood pressure. Middle curve spleen volume. Lower curve time and base line of blood pressure. Time, 1 sec.

change is rather of a passive nature. According to Roy (2) the plethysmographic tracing of the spleen shows specific waves, each of nearly one minute's duration, and the individual heart beats do not appear in the tracing.

From this and other data, such as the fact that compression or even clamping of the abdominal aorta has very little effect on the spleen volume, he assumed that the path of communication between the spleen and the general circulation, as already stated, is narrow and that the spleen volume is relatively small or, at least, not very readily affected by the change in blood-pressure. He therefore regarded the contraction of the spleen as being due to the direct effect of the vagus. If it could be shown that the spleen volume is affected by the heart beat, Roy's argument would become much less cogent, and this has actually been done by Schafer and Moore, who found that fine vibrations on their tracings were obviously due to the heart beat. Although this could not be confirmed in my own tracings, I have reasons for believing that the spleen volume may be influenced, as mentioned above, by changes in blood-pressure.

After an intravenous injection of pilocarpine (1 or 2 c.c. of 1 p.c.



solution) the blood-pressure shows a typical fall with a simultaneous decrease in the spleen volume. If the dose of pilocarpine be large, the blood-pressure remains at this lowered level for a considerable time, the spleen remaining contracted as long as the low pressure persists, a fact which seems to point to the passive nature of the diminution of volume. Under favourable conditions both the change in blood-pressure and spleen volume produced by vagus stimulation could be brought about by an appropriate dose of pilocarpine (Fig 3). In this tracing the

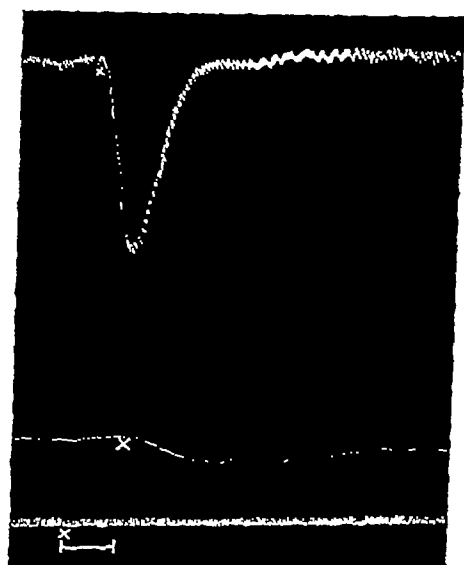


Fig 3 Pilocarpine injection and its effect on the spleen. 1 c.c. of 1 p.c pilocarpine hydrochloride injected into femoral vein at X. Upper curve blood pressure. Middle curve spleen volume. Lower curve time and base line of blood pressure. Time, 1 sec.

passive nature of the change is naturally not so obvious as in the case of the larger dose, but if the findings are correct in that case, why should they not also be correct in the case where the spleen curve shows a closer resemblance to that produced by vagus stimulation?

In order to confirm the passive nature of this spleen contraction, two methods were adopted. In the first place, an attempt was made to eliminate the effect of blood-pressure by the use of Roberts's compensator(7). As, according to Roy(8) and others, the saline solution, especially in large amount, may itself cause dilatation of the spleen, de-

fibrinated blood was used instead of Ringer's solution, and the compensator was accordingly modified to economise the blood. By inserting a cannula into the abdominal aorta and connecting this with the compensator, the blood-pressure could be kept practically unchanged even under vagus stimulation and no contraction of the spleen could be observed (Fig 4)

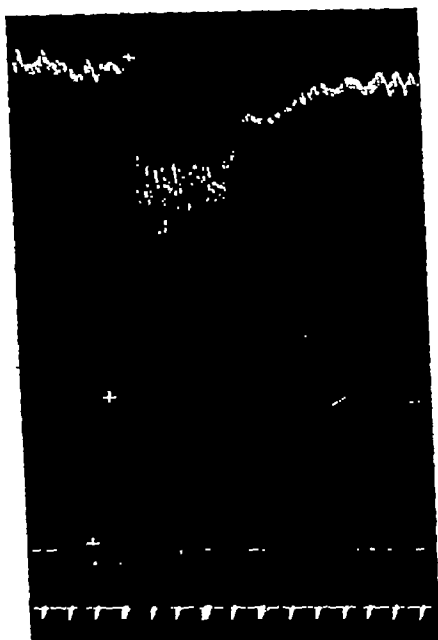


Fig 4a



Fig 4b

Fig 4. Effect of blood pressure on spleen volume. Upper curve blood pressure. Middle curve spleen volume. Lower curve signal and base line of blood pressure. Time, 10 secs.

(a) Stimulation of peripheral end of right vagus.

(b) Blood pressure compensator connected and the peripheral end of vagus stimulated.

Three minutes interval between (a) and (b)

In the second place, the vagus was stimulated inside the thorax below the point at which the nerve sends out its cardiac branch. In no case could even the slightest contraction of the organ be seen, whereas it is quite obvious in the same animal if a fall of blood-pressure is produced by stimulating the vagus in the neck, in spite of the fact that the

nerve has been cut inside the thorax, thus preventing the transmission of any impulse to the spleen Skramlik and Duran-Cao<sup>(3)</sup> obtained similarly negative results and also made other interesting observations. According to them, the stimulation of the peripheral end of the vagus inside the thorax has no effect on the spleen volume, and further, if the vagus and the splanchnic be stimulated simultaneously, the splanchnic alone takes effect, while if the spleen be previously made to contract by stimulating the splanchnic and the vagus be then stimulated before the splanchnic effect dies away, the spleen begins to dilate more quickly than it might otherwise do. From this they conclude that the vagus dilates the spleen only when this organ is previously contracted by the effect of its antagonist, the sympathetic. In this way they thought to have proved that there still exists a certain antagonism between the vagus and the sympathetic in their action on the spleen. As plethysmography has some disadvantages, such as the exposure of the organ for too long, compression of the vessels, etc., they did not employ this method but simply observed the change in volume and colour of the organ, as some other workers have done. If the contraction takes place, the dark red colour is turned to light red and the smooth surface becomes granulated, which latter phenomenon is denoted by them as "Inselbildung". For instance, they observed in one case that the spleen contracted within 3.5 sec. of stimulating the great splanchnic nerve, Inselbildung occurred after 17 secs. and lasted 70 secs., the spleen becoming normal again after 105 secs. In another case in which the splanchnic and the vagus were stimulated successively, the spleen began to contract after 3.4 secs., Inselbildung occurred after 20 secs. and lasted only 30 secs., total recovery taking place after 58 secs. Thus the time of recovery was reduced to nearly one-half. I have tried to repeat this observation but in no case have I succeeded in confirming their result. The results of one of my experiments are given below.

The whole course of the change in volume is given in Fig. 5. There is clearly no difference between these two cases, and in two or three other experiments I obtained the same negative results<sup>1</sup>.

At least two factors come into question in considering the cause of this discrepancy. In the first place, the methods used were different. It is true that the plethysmographic method has disadvantages, as Skramlik and Duran-Cao indicate, and these disadvantages may

<sup>1</sup> After this research was completed, Dr. Hoet, working in the Cambridge Pharmacological Laboratory on the same subject, told me that he had obtained similar results to mine.

Feb 26, 1926. Cat No 24. Under urethane and R.C. mixture and artificial respiration the thorax was opened and the vagus and the large splanchnic nerve in the left side made ready for stimulation. The spleen was put into the spleen box. Blood pressure recorded in the right carotid.

Time (secs.)	Stimulation	B.P. mm. Hg.	Remarks
0	Splanchnicus maj of left side	157	
5		160	B.P. begins to rise
7		162	Spleen begins to contract
15		190	B.P. reaches maximum
39		175	Max. contraction of spleen. Decrease in volume of about 2 c.c.
41	Stimulation off	175	Spleen volume shows little variation
105		162	Volume begins to increase
175		160	Volume increase 1.4 c.c. Still increasing but more slowly
295		150	Recovery of volume almost complete
After 2 minutes			
0	Splanchnicus maj of left side	151	
3		153	B.P. begins to rise
7		154	Spleen begins to contract
26		181	B.P. reaches maximum
42	Stimulation off	166	
52	Left vagus	160	
58		157	Spleen contraction maximum. Decrease of volume 1.8 c.c.
87	Stimulation off	155	Spleen remains contracted
107		155	Spleen begins to dilate
207		158	Increase of volume 1.5 c.c. Still slowly increasing
300		152	Recovery almost complete

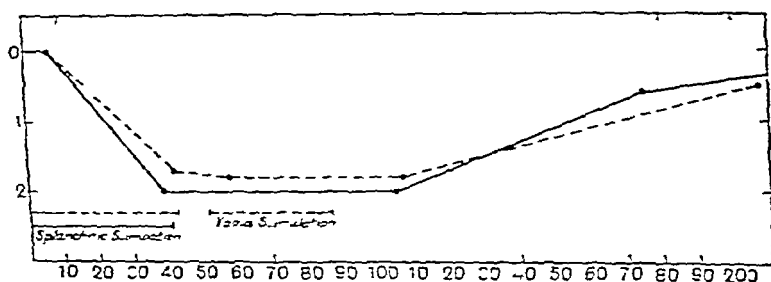


Fig. 5 Effect of splanchnic and vagus on spleen volume.

Splanchnic stimulation followed by vagus stimulation.

— Splanchnic stimulation alone.

Abscissa time in seconds. Ordinate spleen volume in c.c.

0 initial volume of spleen.

be considerable if great care is not exercised quite often no definite results are obtained, especially if the animal happens to be weakly. Taking all these drawbacks into account however it is scarcely credible

that my plethysmograph is not sensitive enough to record a change so evident as to shorten the time of recovery to one-half

In the second place, cats were used in my experiments, while Skramlik and Duran-Cao always used dogs. I do not know to what extent this difference may affect the results obtained

Taking all this into consideration, and with some reserve, I would say that the stimulation of the peripheral end of the vagus, even when it follows the sympathetic impulse, has no effect upon the spleen whatever

(2) *Stimulation of the central end of the vagus* Bulgak(1) classified the nerves going to the spleen into two groups, namely, the motor and the sensory nerves. In the case of the former, contraction of the organ is manifested only on peripheral stimulation, while in the case of the latter it is shown only when stimulation is applied centrally. In the case of the vagus nerve he observed no contraction on stimulating either the peripheral or the central end. He thus excluded the vagus entirely from the list of the splenic nerves. Roy(2), on the contrary, always observed contraction of the organ on stimulation of either end of the vagus. Thus he found that stimulation of the central end of the vagus on one side, the other side being severed for the sake of eliminating the cardiac effect, was always followed by more or less marked contraction of the organ. Comparing this result with that obtained by stimulating the sciatic nerve, he regarded these two as analogous and thought that in this case the vagus was acting merely as a sensory nerve. Repeated trials on my part to obtain this effect resulted to my astonishment not in contraction but invariably in a more or less marked dilatation of the organ. At the same time a gradual fall in blood-pressure was observed (Fig. 6). This fall is typical of the action of the depressor nerve and led me to suppose that this dilatation was also due to the same cause. And such has actually been shown to be the case by Bayliss<sup>1</sup> who, using rabbits in which the depressor is running as an independent nerve, observed this same dilatation of the spleen, the only difference being that there was no after-contraction such as I observed in the spleen of cats (see below). Here again the change in volume of the spleen occurs a few seconds after the fall in blood-pressure. When stimulation ceases, the blood-pressure returns gradually to its initial height, the spleen

<sup>1</sup> This experiment is not described in any of Bayliss's writings, nor indeed elsewhere, but the tracing is given in Starling, *Principles of Human Physiology*, to illustrate another subject and was unknown to me when this work was being carried out. Prof. Starling informed me that the tracing was given to him personally by Bayliss.

contracts simultaneously, and there follow further several intensified wave-movements. If the stimulation be strong or continued longer, say

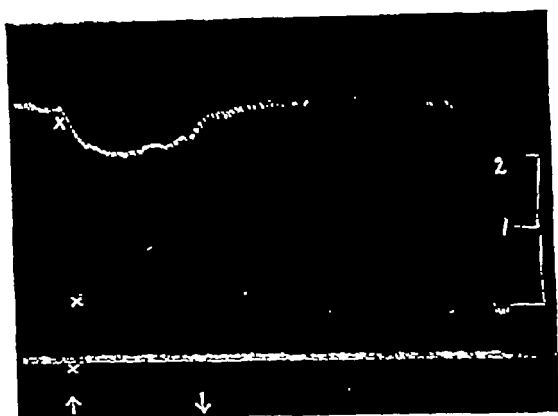


Fig 6 Stimulation of central end of vagus. Upper curve blood pressure. Middle curve spleen volume. Lower curve time and base line of blood pressure. Time, 1 sec.

more than one minute, the blood-pressure rises higher than its initial height when the stimulation is over and the spleen shows also strong after-contraction, so that it becomes for a short time far smaller in volume than it was before the stimulation. In any case the wave-movements following this are generally more obviously intensified than is the case after stimulation of the peripheral end. In comparing this with the effect of stimulation of the sympathetic a marked resemblance is evident, and very possibly this after-effect is due to the secondary action of the increased secretion of adrenaline.

Bayliss(8) obtained similar results also in the submaxillary gland of cats. He cut out the sympathetic going to the gland on one side, leaving the chorda tympani intact, and stimulated the central end of the vagus of the other side. In this way he observed the typical fall in blood-pressure, increased outflow of blood from the gland, and also increased secretion of saliva. Considering that this result might be due to the action of the depressor nerve, he made further experiments to verify it. In cats the depressor fibres are said to run in the left side both in the vagus and in the sympathetic, while in the right side the vagus only contains them. He therefore took out these nerves on both sides and stimulated them separately, and observed in this way that stimulation of the central end of the right vagus, left vagus and sympathetic provoked the fall of blood-pressure and the increase of both

blood outflow and salivary secretion, while stimulation of the right sympathetic produced no result but a slight rise in blood-pressure. These responses are more marked in the case of the right vagus than in that of the left vagus and the sympathetic together. This shows that more depressor fibres are contained in the right vagus than in any one of the remaining nerves. Moreover, he observed that the increase in the outflow of blood is proportional to the depressor effect. In control experiments on rabbits, in which the depressor runs, as already stated above, not in the vagus trunk but as an independent nerve, he confirmed the view that these effects are really due to the action of the depressor nerve.

Does such a relation exist between the stimulation of the vagus and the spleen volume? If such is proved, then we obtain a further proof in support of the view that this dilatation of the spleen is due to the depressor action, although it has already been confirmed by Bayliss himself in rabbits. In order to establish this point I took these four nerves as he did, and stimulated their central end separately. The result was exactly as was anticipated: the stimulation of the right vagus caused most conspicuous dilatation, less evident were the results of stimulating the left vagus or sympathetic, while the right sympathetic gave no result of this kind at all.

Moreover, the dilatation provoked by the right vagus was nearly equal to the sum of those by the left vagus and sympathetic. In other words, the depressor fibres are pretty evenly distributed in both sides.

The examples quoted below will show this relation.

Exp 1 1 II 1926 The vagus and the sympathetic of both sides in the neck were prepared for stimulation. The central end of them was stimulated separately, the current being of the same strength. The results obtained are tabulated as follows.

	R. vagus	L. sympath	L. vagus	L. vagus + L. sympath.
Increase of vol	(i) 6 c.c. (ii) 6 c.c. <hr/> 6 c.c.	2 c.c.	5 c.c.	6 c.c.
Decrease of vol (after effect)	(i) 13 c.c. (ii) 8 c.c. <hr/> 11 c.c.	5 c.c.	7 c.c.	12 c.c.

Exp 2 8 II 1926 The same kind of experiment was done. The results are as follows.

	R. vagus	L. sympath	L. vagus	L. vagus + L. sympath
Increase of vol	(i) 15 c.c. (ii) 13 c.c.	7 c.c. 11 c.c.	8 c.c. 9 c.c.	— 13 c.c.

These results undoubtedly show that what has been found by Bayliss in the submaxillary gland, is exactly applicable to the spleen, and in my opinion this is sufficient evidence that the stimulation of the central end of the vagus causes in cats the dilatation of the spleen, which action is duly attributable to the effect of the depressor nerve. However, in some cases, if the strength of the stimulation and the sensibility of the animal are brought into such a relation as to cause a strong sensory impulse without the depressor effect, then such a contraction as Roy observed might be seen. In this case there is of course no fall but rather a rise in blood-pressure. Therefore Roy's view that the vagus acts as a sensory nerve is still to a certain extent correct, especially since, in the tracing given by Bayliss(8) taken from rabbits in which the depressor nerve but not the vagus was stimulated, only the dilatation is seen and no after-contraction at all.

#### SUMMARY

1 The stimulation of the peripheral end of the vagus in the neck causes a diminution in the spleen volume. But if the fall of the blood-pressure is prevented by means of a blood-pressure compensator, or if the vagus is stimulated inside the thorax below the point at which the nerve sends out its cardiac branch so that no considerable change in blood-pressure occurs, the stimulation does not provoke any change in the spleen volume at all. Moreover, the spleen volume varies with the blood-pressure. Therefore this shrinkage of the spleen is merely a passive effect due to the fall of the blood-pressure.

2 The stimulation of the central end of the vagus provokes the dilatation of the spleen. This dilatation is to be ascribed to the stimulation of the depressor fibres, which in cats are contained in the vagus trunk in the right side and both in the vagus and the sympathetic in the left side. The distribution of the fibres is nearly equal in both sides. Therefore the effect evoked by the right vagus is nearly as strong as the sum of those by the vagus and the sympathetic respectively of the left side.

I desire to express my gratitude to the late Prof. Langley, who kindly gave me the privilege of working in his laboratory, and my sincere regret for his death. My thanks are also due to Prof. Barcroft for his suggestions, encouragement and help continuously given to me throughout the whole course of this research.



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# STUDIES ON THE INTERNAL SECRETIONS OF THE OVARY III The effects of injection of œstrin during lactation

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## I INTRODUCTION

IN a previous paper(7) it was shown that the œstrus inhibiting function of the persistent corpus luteum of pregnancy may be overridden by injection of œstrin<sup>1</sup>, and the significance of this fact was also discussed. In the present communication the effects of injection of œstrin during lactation, when a state of prolonged diœstrus is normally found in the mouse(8) and in the rat(4), is dealt with. Hitherto, the evidence that the persistent corpora lutea of lactation which are found in these animals(4) are responsible for the non-occurrence of œstrus has not been so definite as in the case of the persistent corpora lutea of pregnancy. Functional correlation, however, strongly suggests some such mechanism and it is hoped to show fairly definitely in the present paper that the non-occurrence of œstrus during the suckling of a litter of more than two is due to the persistence of the corpora lutea resulting from the immediate post-partum ovulation. This whole question will be discussed in some detail later, but it may be said here that the *a priori* idea behind these experiments was that the injection of a fairly large unitage of œstrin might override the persistent corpora lutea, and lead to the abnormal occurrence of œstrus during lactation.

Later, when it was found that large doses were required, some other experiments, which will also be recorded here, were started on the effects of large doses on the non-suckling ovariectomised mouse. Still later in this series of experiments the effects of injection into suckling ovariectomised mice were investigated.

## II METHODS AND MATERIALS

Little new in the way of methods and materials needs to be described. The preparation of the extracts, and the detection of the œstrous cycle

<sup>1</sup> Elsewhere suggested as a convenient term for the œstrus producing hormone of the ovary

and pregnancy as applied to this work were described in Part II of this series. Points specially relevant to this present paper are dealt with below.

*Source of œstrin* The method of preparation has been the same as previously described, but more diverse material has been used as a source. It has, of course, been reported frequently that the œstrus producing hormone can be extracted from placenta, but the greater initial purity of ovarian extracts makes the use of ovaries preferable for experimental work. The use of placenta as a source is, however, far less expensive, and practically all the material required for the experiments described in this paper was obtained from a mixed batch of ovaries and placenta. The crude production had a comparatively poor activity per gram, but purification with methyl alcohol at 0° C resulted in a product sufficiently pure to make an emulsion giving 20 m u (mouse units) per c c. This product, when tested, was kept in cold store and emulsified in small quantities from time to time as required.

*Method of injection* In the experiments on injection during pregnancy the doses given were spread over a number of days, but in the present series the whole amount to be given was injected at one time. The advantages of this were twofold: (a) in the absence of any information as to how long œstrin will remain intact in the body, injection at one time afforded a better index of the effective dosage, and (b) it was possible to obtain a more accurate time relation. After these experiments were begun, however, Evans and Burr<sup>(1)</sup> reported that a given amount is more efficient when administered in a number of injections. After injection vaginal examinations were made until such time as œstrus appeared and passed off, or if œstrus failed to appear until such time as all probability of it appearing had vanished.

*Time of injection* Since it seemed not impossible that the susceptibility to an extraneous supply of œstrin might vary during the three weeks of diœstrus during lactation, it was endeavoured to make the injections at approximately the same stage of lactation. The actual time arrived at was the tenth day after parturition when the corpora lutea of lactation are presumably at maturity. In some few cases injection took place a little either side of this stage, but all the injections were made between the eighth and twelfth day of lactation.

*Weighing of young* Where it was desired to ascertain the effects of injection of the suckling mother on the growth curve of the young, daily weighing of the litter was made during the whole of the suckling period of three weeks. From the total weight, the average weight of

the individuals each day was arrived at. The same system had been used previously (5) in working out the growth curves of normal young, and the control growth curves were, therefore, already in existence. In the normal growth curves it had been found that the number in the litter suckling was a very potent factor in determining the individual growth curve, and for this reason it is essential to compare the growth curves of experimental young with those for normal animals of the same size of litter.

*Mating of suckling females* In cases where an artificial oestrous period was induced during the dioestrus which normally occurs during lactation, it was desirable to ascertain whether or not ovulation took place at the same time and, furthermore, to ascertain whether the female would become pregnant. This involved the mating of the suckling females, and this was found to present one of the most awkward practical problems associated with the work. Since eating of some of the young by either the male or female nearly always took place, and since it was clearly undesirable to have part of the litter eaten as soon as the injection was made, the mating was not made until oestrous symptoms were observed. If copulation took place the litter was immediately removed so that an optimum chance of pregnancy ensuing was provided. (See Kirkham (3) for discussion of the inhibitory effect on implantation of suckling.) This procedure meant that animals used for mating at the induced oestrous period could not be used for testing the effect on the growth curve of the young.

### III EFFECTS OF LARGE DOSES OF OESTRIN

When it became clear that large doses of oestrin were required to produce oestrous symptoms in animals suckling a big litter, it was thought desirable to investigate the effects of giving large doses to non-suckling ovariectomised mice. From the routine testing of extracts it had been noticed that

(a) The least dose required to produce oestrous symptoms (*i.e.* 1 m.u.) produced cornification lasting in more than half the cases for one day only. In a smaller percentage of tests cornification resulting from the administration of one mouse unit was observed to last over two consecutive daily examinations, namely, between 1 and 2 days. One mouse unit was very rarely found to produce more prolonged cornification than this.

(b) Administration of heavy doses, however, produced cornification lasting over many days.

It was decided, therefore, to do a fairly large number of injections

of known quantities of oestrin into ordinary ovariectomised mice with a view to ascertaining what correlation, if any, existed between the number of mouse units injected, and the duration of the cornification produced. At least one injection was made of every dose between 2 and 20 m u, and the 105 injections made in all gave the following correlation table

TABLE I. Correlation table for number of mouse units injected and days of oestrus produced.

		Mouse Units																			
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Days of Cornification	1	6																			6
	2	17	1	1																	19
	3	6	5	1	4	1															17
	4	1	5	1	1	4	1	2	1												18
	5		2	1	2	1	2	1	2	2		1				1					15
	6						1	1	1	1	1			1							6
	7									1	1		1	1	1			1	1		7
	8										1	1		1	1	2					6
	9								1			1		1			1		1		5
	10											1			1		1				3
	11																	1		1	2
	12																	1		1	2
	13																	1			1
		30	13	4	7	6	4	4	4	5	3	4	1	4	3	3	3	3	2	2	105

Superficial examination of this table shows a very definite positive correlation between the number of mouse units injected and the duration of cornification. The following remarks may be made relative to this table

(1) The coefficient of correlation is  $904 \pm 012$ , which indicates a very marked relation between mouse units injected and length of resulting oestrus

(2) Since the difference between  $r = 904$  and  $n = 934$  is barely significant, the relation between the mouse units and days of oestrus may be regarded as linear

(3) The average variability for the result of the injection of a given number of mouse units is represented by a standard deviation in length of oestrus produced of 99 days

#### IV INJECTION DURING LACTATION

##### (a) Effect on recurrence of oestrus

As was pointed out in a previous paper (6), oestrus occurs in the mouse within 24 hours of parturition. In the absence of suckling after parturition, oestrus recurs after a period corresponding to the normal length of

the œstrous cycle, so it must be assumed that the fact of parturition having taken place does not further disturb the œstrous cycle. If, however, suckling takes place no œstrous symptoms are normally experienced for some three weeks after the immediate post-partum period. This suggests that the fact of suckling taking place transforms the corpora lutea of the immediate post-partum ovulation into persistent corpora lutea with an œstrus inhibiting function<sup>1</sup>.

Further, since prolonged suckling of the same or of a foster litter does not further postpone œstrus it may be supposed that the greatest duration of the functional life of the persistent corpus luteum of lactation in the mouse is little more than three weeks.

A detailed account of the effects of lactation on the œstrous cycle in the mouse is to be found in the previous paper (Parkes(6)), but it is of importance to note here that it was previously found that the suckling of litters of one or two only did not produce the inhibitory effects of suckling larger numbers of young. No satisfactory explanation of this was arrived at, but when injections of œstrin during lactation were started it was thought from this fact that females suckling large litters might require a greater dose to produce artificial œstrus than animals suckling fewer young.

*Dosage of œstrin necessary to produce œstrus* The fewness of litters of 8 and 9 born precluded much work being done with these sizes of litter suckling, but for all sizes between 3 and 7 the least number of mouse units of œstrin sufficient to override the persistent corpus luteum of lactation and produce an artificial œstrous period has been determined. The experimental records are given in Table II below.

In these experiments the procedure was to start with a low dosage for each litter size and work upwards until a dose was found which would bring the doe into œstrus, each injection being made into a different animal. In all 37 injections were made into females suckling various sizes of litter before the minimum dose for each size of litter up to and including litters of 7 was determined. In addition, untreated does suckling litters of 2-3-4 were again examined for the occurrence of œstrus during suckling, and it was again found that no untreated animal suckling more than two young comes into œstrus during this period. The whole of these results are shown diagrammatically in Fig 1 below.

<sup>1</sup> The full evidence that the persistent corpus luteum has an œstrus inhibiting action is not discussed in this or in the previous paper dealing with the persistent corpus luteum during pregnancy. It is hoped to discuss this fully in a later paper relating to the preparation of œstrus inhibiting extracts from corpus luteum.

TABLE II Mouse units injected according to size of litter suckling

No of animal	No being suckled	No born	Stage of lactation when injected	M.U given	Result
LVS	2	8	No injection	0	+
142 b	2	9	"	0	+
140	3	5	"	0	-
139	3	11	10 days	2	-
178	3	4	10 "	3	-
182	3	8	9 "	4	+
142 a	4	4	No injection	0	-
154	4	4	11 days	1	-
160	4	7	10 "	2	-
138	4	10	10 "	3	-
149	4	8	9 "	4	-
133	4	4	10 "	5	+
152	5	5	10 "	1	-
132	5	5	11 "	3	-
157	5	8	10 "	3	-
146	5	5	12 "	4	-
150	5	6	9 "	4	-
125	5	5	12 "	5	-
130	5	4	11 "	6	+
137	5	8	10 "	7	+
128	5	6	10 "	8	+
166	6	6	10 "	2	-
159	6	7	10 "	3	-
131	6	6	11 "	3	-
155	6	6	10 "	4	-
122	6	8	12 "	5	-
126	6	9	11 "	5	-
136	6	8	11 "	6	-
156	6	6	10 "	7	-
163	6	8	11 "	8	-
173	6	6	9 "	9	+
127	6	6	10 "	10	+
153	7	8	10 "	1	-
158	7	9	10 "	4	-
129	7	8	8 "	5	-
141	7	10	10 "	6	-
135	7	7	10 "	7	-
148	7	7	8 "	8	-
136	7	9	10 "	9	-
183	7	9	9 "	10	+
176	7	8	9 "	11	+

In Fig 1 points at which observations were made are shown by circles, positive results being solid and negative results hollow circles. Since two young only suckling are not sufficient to inhibit œstrus the minimum dose of œstrin required must be considered in relation to the number of young suckling in excess of two, and it will be seen from the above diagram that the number of mouse units required is roughly equal to twice the number of young in excess of two. In other words, the number of mouse units required to produce œstrus during lactation

is  $2(n-2)$ , where  $n$  is the number of young suckling. The approximation to this equation is only rough, the points for litters of three do not agree,

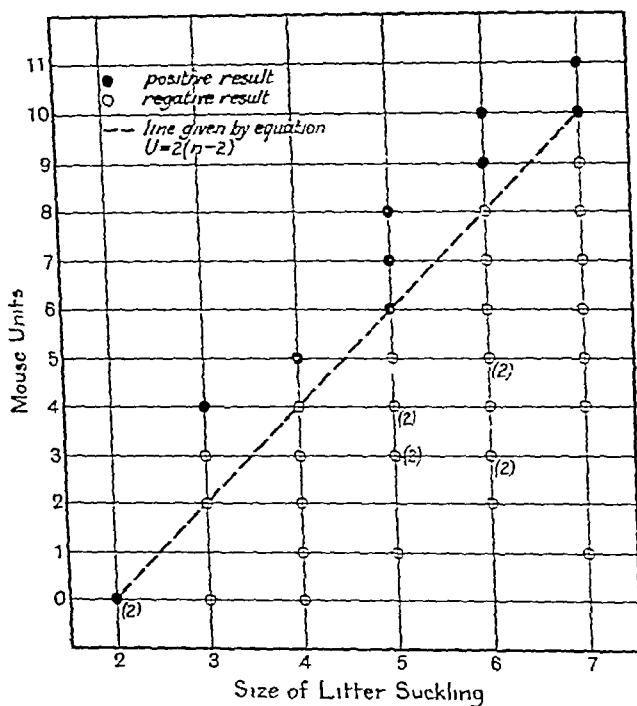


Fig 1 Number of mouse units required to produce œstrus during lactation.

for instance, but, nevertheless, the line given by the equation does represent the general nature of the results

It was at first thought that the real significance of these results might lie in the number of mouse units required being correlated with the number born, and only superficially with the number suckling (the number born being in most cases the same as the number suckling). With this in view cases were prepared where the two numbers were different, and these cases show conclusively that the number suckling, not the number born, is the crucial factor. The two females suckling only two each, for instance, came into œstrus in spite of the fact that they had had respectively litters of eight and nine born.

Why each additional young suckling should be able to nullify the injection of two additional mouse units is not at the moment obvious, but it is clearly only an extension of the problem of how lactation inhibits œstrus and will be dealt with in Section V.



*Does ovulation occur at the artificial œstrus period?* The records given above make it clear that changes analogous to those characteristic of normal œstrus can be produced in the accessory organs during lactation by the administration of sufficient amounts of œstrin. In addition, in cases where the injected suckling animal was mated, copulation invariably took place if the injection was sufficient to lead to the characteristic changes in the vagina. Since copulation will only take place at œstrus this fact has three interesting implications.

(a) Additional evidence is provided that the cornification of the vagina as detected by the vaginal smear technique is a reliable criterion of œstrus.

(b) Since copulation only took place when cornification was observed after injection, it is shown that the minimum amount of œstrin required to produce cornification during normal diœstrus is also the minimum amount which will give rise to the psychic symptoms resulting in copulation.

(c) It is also evident that the induced or artificial œstrous period brought about during lactation by injection of œstrin is a real and genuine period accompanied by all the normal non-ovarian symptoms.

The next question to consider is as to whether or not these induced periods of œstrus are completed by the recurrence of ovulation. This problem was approached by (a) the observation of the results of copulation at the induced period, and (b) by the histological examination of ovaries after injection. If ovulation had taken place at these periods it is reasonable to assume that pregnancy would follow copulation in at least a proportion of cases, especially in view of the fact that females used for this test had the litters removed after copulation. Five animals, outside the series recorded above, were used for this purpose, and heavy doses were given to ensure that œstrus would occur. Mating took place the day following the injections. The results were as follows.

TABLE III. Results of copulation at the induced œstrous period

No of animal	Stage of lactation	No born	M.U given	Result
134	8 days	7	15	œstrous symptoms and copulation, but no pregnancy
180	10 "	5	10	do
181	10 "	4	10	do
185	9 "	6	12	do
187	10 "	6	12	do

These results suggest that ovulation does not take place at the induced œstrous period. This conclusion was supported by the histo-

logical examination of the ovaries of further animals treated in the same way but killed after copulation, and is in keeping with the results previously reported for the injection of œstrin during pregnancy

*(b) Effect on lactation*

Since œstrus and lactation are incompatible for at least three weeks after parturition, excluding the immediate post-partum period, it seemed possible that the artificial production of œstrus during this time might adversely affect the lactating powers of the female. The intensity of lactation can only be judged indirectly in an animal such as the mouse, but for at least 17 days the young are entirely dependent on the activity of the mammary glands of the mother, and their growth indicates the efficiency of the mother in this respect. It was, therefore, decided to study the growth curves of the young suckled by does artificially brought into œstrus. The growth of young mice under normal conditions in the colony had already been fairly extensively investigated (5), and provided good data with which the growth curves of the experimental young could be compared.

The growth curve of the young mouse during the suckling period consists of three distinct phases. The first is one of steady growth and lasts for about 10 days. Following this, there is a slackening off in the rate of growth, and during this phase both the gross and percentage daily increment fall considerably. At about 17 days the third phase, characterised by very rapid growth, sets in, and, though the percentage increment never becomes as high as during the first week, the gross increment increases again. During the last 3-4 days of this third phase the young begin to eat for themselves.

As might be expected, growth is very much more rapid when a small litter only is being suckled, the individual gross and percentage increments are much reduced where the litter is large. This necessitates a normal growth curve being made for each size of litter.

From the point of view of the lactation experiments it is unfortunate that the œstrin injections had to be made just when the growth of the young was slackening for other reasons. Injection of the mother during the first phase of rapid growth might have yielded more striking results. Even so, however, where a considerable mouse unitage was injected into the mother at 8th-12th day of lactation, the growth of the young was much retarded compared with the normal growth.

The following diagram shows the growth curves of certain of the experimental litters compared with normal curves for the corresponding

size of litter The arrows indicate the time at which the injection of the mother took place, and it will be seen that in each case the normal and experimental curves follow the same course to this point, after which the experimental curves definitely drop below the normal

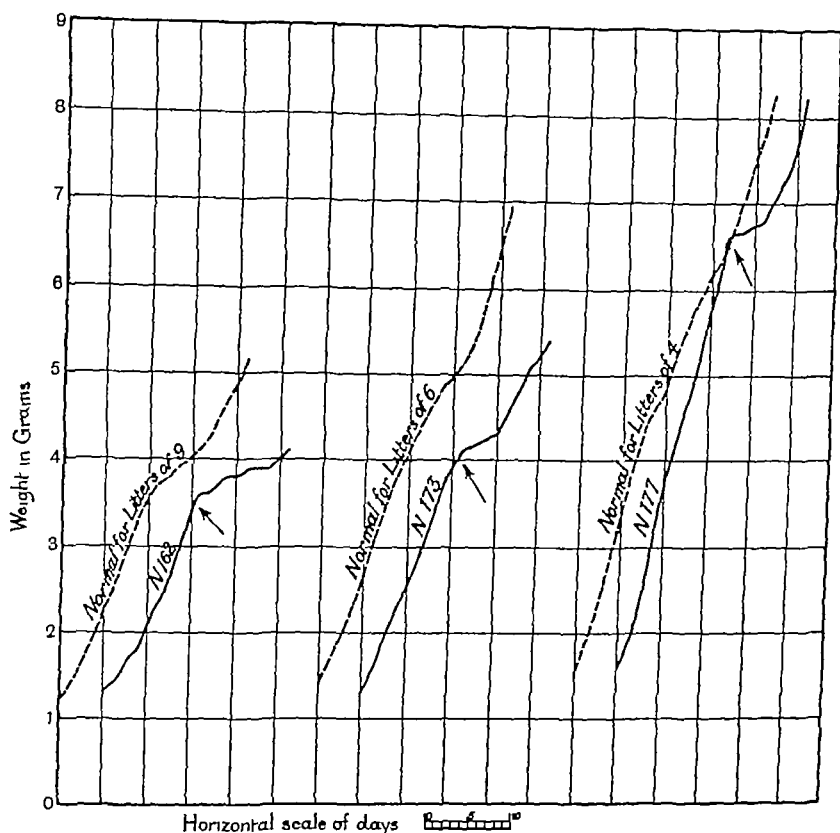


Fig 2 Growth curves of young of normal and injected mothers

All of these three does had a sufficient dosage of œstrin to produce œstrus N 162 had 12 m u, N 173 9 m u and N 177 10 m u The same check in the growth of the young was observed to a lesser degree where the injections were not quite sufficient to produce œstrous changes There seems little reason to doubt that this check is a real effect of injection, and the fact serves to emphasise still further the reciprocal correlation between ovaries and mammary gland At the same time it is possible that the increased metabolism associated with œstrus might in itself have an adverse effect upon lactation

## V THE MECHANISM OF THE ŒSTRUS INHIBITION SET UP BY LACTATION

The fact that œstrus does not occur during lactation in either the rat or the mouse is usually ascribed to the fact that lactation has resulted in the corpora lutea of the immediate post-partum ovulation becoming persistent. Long and Evans(4) in the rat for instance, showed that removal of the young in the middle of the suckling period resulted first in degenerative changes in the corpora lutea and then in the appearance of œstrous symptoms. Why the suckling of less than three young does not have the same effect of making the corpora lutea persist and inhibit œstrus is not clear, but it suggests some purely quantitative mechanism such as Hammond(2) has suggested in the regulation of the œstrous cycle. Some such supposition is also pointed to by the correlation shown above between the number of mouse units necessary to produce œstrus and the number of young suckling. Once the corpora lutea have become persistent it is extremely difficult to see why they should be more potent when more young are suckling. It may be emphasised again that the number born is of no importance, the number suckling is the vital factor. Also it is difficult to see why the corpora lutea of lactation can, in certain circumstances, withstand up to 10 m u., where the persistent corpora lutea of pregnancy are overridden in many cases by 5 m u. (7). These facts lead to the consideration of the possibility that the control of the history of the corpus luteum by the mammary gland might be more apparent than real, and that the inhibitory effect of lactation might be merely a question of maternal exhaustion<sup>1</sup>. If this were so the histological persistence of the corpora lutea could be explained on the grounds of the absence of œstrous stimulus.

In addition since œstrin is fat soluble, the further possibility arose that the excessive lactation of the mouse might actually drain away a certain amount of the hormone, and account (1) for the normal occurrence that one or two young have no inhibiting effect, and (2) for the fact that every additional young suckling increased the amount of œstrin which had to be injected to cause œstrous symptoms. Against this conception there is of course the fact recorded in a previous paper(6)

<sup>1</sup> The amount of milk secreted by a lactating mouse is relatively very great. An average sized litter weighs about 40 grm. in all at the time when it ceases to become totally dependent on the mother (i.e. at 18-19 days old). Of this amount at least 30 grm. have been put on during lactation representing some 15 grm. of dry matter. Even if the dry matter excreted by the young is neglected, and even if it be assumed that mouse milk has as much as 20 p.c. dry matter it would seem that the lactating mother must produce 75 grm. of milk in 18 days, or 4 grm. per day. This represents  $\frac{1}{4}$  of its own weight per day. On the same basis a cow would produce 2 cwt. of milk per day.

that prolonged suckling of foster litters does not further inhibit œstrus after the involution of the corpora lutea of lactation, but nevertheless the possibility appeared to be worthy of further consideration

The whole question comes down to this Does lactation inhibit œstrus by an influence exerted on the ovary, and hence presumably through the corpora lutea, or by some other means?

The experimental answering of this question has been comparatively simple If the inhibition is set up through the ovary, then the removal of the ovaries should stop the inhibition, while if some other mechanism is concerned removal of the ovaries should not inhibit the effect of lactation in nullifying the injection of heavy doses of œstrin In other words, does a lactating ovariectomised mouse require more œstrin to produce œstrous symptoms than a non-suckling ovariectomised mouse? To answer this question a number of mice were ovariectomised soon after parturition and subsequently injected while still suckling The results are summarised in the following table

TABLE IV Injection of ovariectomised suckling mice

Mouse	No born	Ovariectomy (days after parturition)	Injection (days after parturition)	No suckling	Mouse units	Result
N 208	4	5	9	3	1	Positive
N 209	5	6	10	3	2	"
N 211	4	5	8	4	1	"
N 194	5	2	8	4	2	"
N 202	10	8	12	5	1	"
N 212	5	4	8	5	2	"
N 213	8	4	8	5	3	"
N 190	6	6	12	6	1	Negative
N 193	7	3	9	6	2	Positive
N 195	7	3	9	6	3	"
N 196	8	3	9	6	4	"
N 191	9	4	10	7	1	Negative
N 174	7	4	8	7	2	Positive
N 175	8	5	9	7	3	"

This table shows no trace of the correlation between mouse units required and number suckling which was demonstrated in the case of the non-ovariectomised mice, two mouse units being sufficient in every case to produce œstrous symptoms This result leads inevitably to the conclusion that, though there may be a slight inhibiting action set up by lactation *per se*, it is able to override no more than 1 m u of œstrin, and hence to the conclusion that the mechanism of the inhibition is dependent upon the ovary and hence presumably dependent on the corpus luteum At the same time these experiments throw no light on the mechanism whereby the functioning of the mammary glands causes

the corpora lutea of ovulation to persist. Some internal secretory mechanism has been suggested, but the evidence is very uncertain.

## VI SUMMARY

(1) A mouse suckling a litter of more than two does not come into œstrus (except for the immediate post-partum period) during normal lactation. This is usually ascribed to the act of lactation causing the corpora lutea of the post-partum ovulation to become persistent and, therefore, œstrus inhibiting.

(2) By injection of the œstrus producing hormone during this time it was found possible to induce œstrus, at which copulation would take place. The amount required, however, showed a close correlation with the number of young suckling. Where litters of seven were suckling as much as ten mouse units of œstrin were required. The minimum amount necessary decreases with the size of litter, until, as already shown, where only two young are suckling no injection is required, œstrus is spontaneous.

(3) Since no pregnancy was observed to follow copulation at the induced œstrous period and since ovaries sectioned after copulation showed no young corpora lutea, it is concluded that ovulation did not occur during the artificial period.

(4) Mice ovariectomised early in lactation required only small amounts of œstrin (about two mouse units) to produce œstrus, whatever the size of litter suckling, so it is clear that the inhibitory effect of lactation is set up *via* the ovary, and hence presumably *via* the persistent corpus luteum.

(5) These results on the overriding of the œstrus inhibiting persistent corpora lutea of lactation are clearly comparable with the conclusions reached as the result of injection of œstrin during pregnancy (7) but the dosage required to override the corpora lutea of lactation is greater than that required in the case of the corpora lutea of pregnancy.

As in previous work we are much indebted to Dr F. H. A. Marshall, F.R.S., and Professor J. C. Drummond for criticism and advice, and to Dr F. W. R. Brambell for histological assistance.

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# THE OSMOTIC PRESSURE OF THE AQUEOUS HUMOUR AND ITS PHYSIOLOGICAL SIGNIFICANCE

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IN two previous papers<sup>(1)</sup> it has been suggested that the vascular pressures in the eye showed nothing incompatible with the hypothesis that the intra-ocular fluids were formed by dialysation from the blood. The osmotic pressure of the aqueous humour and its value relative to that of the blood is a question of equal importance, and in the consideration of the mechanism of the formation and absorption of this fluid these two inter-related pressures must always be viewed together.

The literature of the subject shows a wide divergence of opinion. Two methods have been employed in the determination of the osmotic pressure of the aqueous humour—a physical method depending on the lowering of the freezing point and a biological one based on the plasmolysis of red blood corpuscles.

The difficulty of the small quantity of fluid available compelled those employing the cryoscopic method to experiment with the collected aqueous humour of many animals, and to compare this with a "typical" sample of serum. The earlier observers consistently arrived at the conclusion that the aqueous was hypertonic to blood serum, the average ratio being about 11:10—Dreser (ox)<sup>(2)</sup>, Kunst (ox)<sup>(3)</sup>, Richon DuVignaud (rabbit)<sup>(4)</sup>, Botazzi and Sturgio (ox)<sup>(5)</sup>, Scalinci (dog)<sup>(6)</sup>. Later, van der Hoeve (ox)<sup>(7)</sup>, finding variations in either direction, pronounced them isotonic, a conclusion corroborated by Osborne (ox)<sup>(8)</sup> while Deiter (rabbit)<sup>(9)</sup>, using a micro-apparatus and comparing the two fluids of the same animal, found in three experiments that the aqueous humour was isotonic in one and hypotonic to the serum in two. Largely owing to technical difficulties the method has therefore given inconclusive results. Further an exact comparison cannot be said to result from the lumping together of the aqueous humour of several animals and the comparison is rendered still more questionable when it is remembered that in the estimation a temperature difference of 0.001° C—a quantity difficult to measure with any approach to accuracy—registers to the quite appreciable variable of 9 mm. Hg.

Using the method of plasmolysis the earlier observers were again united in considering the aqueous humour hypertonic to serum—Hamburger (horse)<sup>(10)</sup>, Kunst<sup>(3)</sup>, Manca and Deganello (oxen)<sup>(11)</sup>—a conclusion supported by Manca<sup>(12)</sup> using the hæmatocrit. Later investigators, however, using the same method, failed to get the same consistent results. Pomer (ox)<sup>(13)</sup> considered that the friability of the red cells varied in itself up to 50 p. c. and he arrived at the same conclusion as Nuel<sup>(14)</sup> and Rissling<sup>(15)</sup> (using various animals) that any differences found were within the limits of the experimental



errors involved in the method, and that the aqueous humour was isotonic with the blood serum "more or less" The method cannot pretend to great accuracy, and in addition to the source of error depending on the variable behaviour of the red cells, there are others depending on the treatment of the serum

From a consideration of these results it would seem apparent that, whatever their exact relationship, the osmotic pressure of the aqueous humour and the blood are not far removed in magnitude, and in comparing values so nearly allied, and in dealing with solutions so widely different in their molecular aggregation, it would seem preferable to employ as far as possible a direct method of measurement. If the aqueous humour is a dialysate, the difference between the osmotic pressure of this fluid and the plasma can be determined by such a method, which is very much more sensitive than those hitherto employed, and will allow the comparison of the two fluids of the same animal to be made under conditions as constant and as near to the normal as experimental manipulations permit.

In the present experiments the total osmotic pressure was divided into two fractions—that due to the colloids and that due to the crystalloids. The osmotic pressure of the former was measured directly by employing a micro-osmometer capable of dealing with the small quantities of fluid available, provided with a membrane impermeable to colloids. Through this membrane the crystalloids could permeate freely, and any variation in their distribution through osmotic interchange was determined by estimating their concentration before the experiment commenced and after equilibrium had been established. These estimations were confined to the aqueous humour, since, being a comparatively simple and dilute solution, the results obtained therein are more readily interpreted than corresponding results obtained in blood. Any change in the total concentration of dissociated salts was determined by electrical conductivity measurements. The aqueous is to all intents and purposes a physiological salt solution and practically protein-free, and it may be taken that the measure of its conductivity under constant conditions provides an index of any change in its salt content. Any change in the concentration of undissociated crystalloids, which are represented largely by sugar, was determined by the chemical estimation of this substance. The difference in osmotic pressure due to the non-diffusible substances was therefore read off directly on a manometer as mm Hg, since the membrane offered no permanent resistance to the passage of diffusible substances and they would therefore pass freely from one side to the other until osmotic equilibrium had been established, a determination

of any change in their distribution would be an index of the difference in the component of the osmotic pressure due to their influence. A summation of these two fractions was taken as providing a relative determination of the total osmotic pressure of the two fluids.

A micro-osmometer was designed as illustrated, and throughout all the experiments it was used immersed in a thermostat kept at  $18^{\circ}\text{C}$

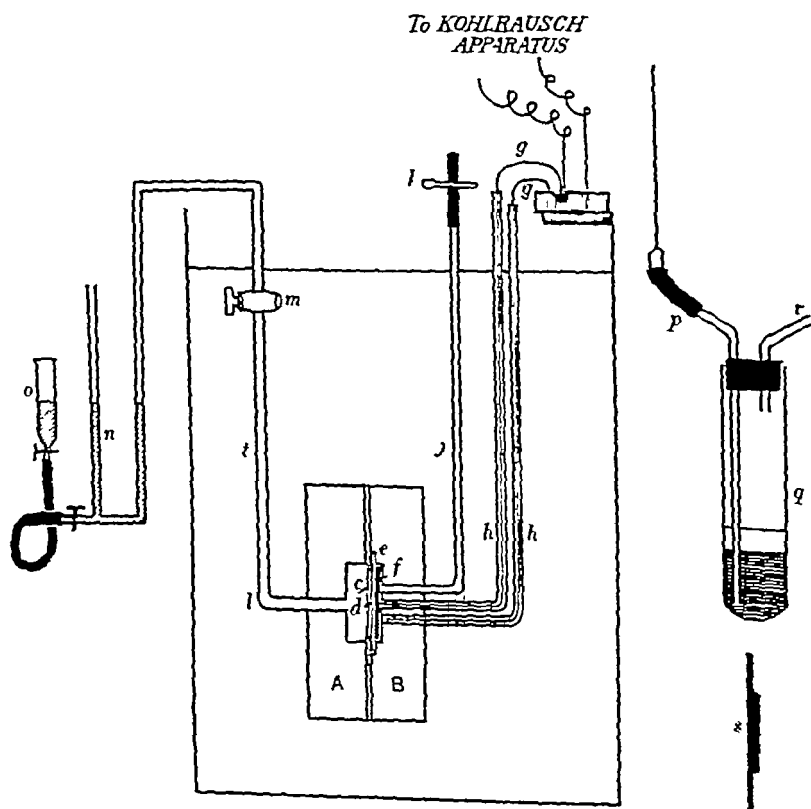


Fig 1

It was made of glass, two plates of which (A and B), with the opposing surfaces accurately ground were clamped together (In the figure the clamp is not shown in position) In each plate a rectangular cell was cut that in A—the “blood cell”—was made of such capacity as to contain 0.5 c.c. of blood ( $1 \times 1 \times 0.5 \text{ cm}$ ) that in B—the “aqueous cell”—to contain 0.2 c.c. of aqueous ( $1 \times 1 \times 0.2 \text{ cm}$ .) This was the size of cell which was found most convenient to work with, as the total quantity of aqueous obtainable from the eye of the average rabbit is from 0.25

to 0.30 c c, and a cell of such size, while being shallow enough to allow osmotic equilibrium to establish itself fairly rapidly, at the same time permitted the spacing of two rectangular electrodes of sufficient area at a sufficient distance apart to ensure reliable conductivity measurements. Between the two a membrane of cellophane<sup>1</sup> was interposed (*c*), supported by a stout copper gauze (*d*), the joint being made water-tight by surrounding the gauze on both sides by a thin rubber washer peripherally, which fitted accurately into a shallow ledge (*e*) cut from the plate (*B*). The washer projected slightly from the sides of the aqueous cell so that the electrodes at no place came into contact with the gauze, and this latter was further insulated by coating it with bitelite, thus preventing any interference with the measurements of the conductivity, as was verified by standardisation with potassium nitrate. Two platinum electrodes (*f*) were cemented on to the parallel sides of the aqueous cell, and led off by platinum wires (*g, g*), cemented into holes running through the thickness of the plate (*B*). These were carried above the level of the surface of the water in the thermostat by glass capillaries (*h, h*) ground and cemented into the plate of the osmometer. Connection was then made through mercury contacts with a Kohlrausch apparatus. From each cell, fitting securely by ground joints into holes running through the thickness of the glass plates, two tubes ran upwards above the water surface: the one (*j*)—the tube from the aqueous cell—terminated in a small rubber tube, which could be opened or closed by a clamp (*k*), the other (*l*)—the tube from the blood cell—could be closed by a stop-cock (*m*), beyond which connection was made to a mercury manometer (*n*) and levelling bulb (*o*).

The majority of the experiments were done on rabbits. The aqueous humour was taken from both eyes and mixed. It was withdrawn under sterile conditions by means of a syringe dried with alcohol and ether. A special needle was made with a broad lance point, and this was introduced into the cornea near the limbus obliquely, such a needle is inserted with less disturbance than the ordinary round pointed instrument, and with the latter it is difficult to prevent the aqueous from escaping round it at the moment of its introduction. 2 p.c. cocaine was instilled into the conjunctival sac as an anæsthetic, it has been repeatedly demonstrated that this procedure does not alter the properties of the intra-

<sup>1</sup> See Verney (*this Journal*, 61 p. 319, 1926), who has tested the permeability of cellophane to crystalloids and its impermeability to serum proteins. I am indebted to Dr Verney for demonstrating his apparatus to me before his work was published from which I have freely borrowed several ideas.

ocular fluids to any appreciable extent. Before the needle was introduced the cornea was dried with blotting paper to avoid contamination by tears. Blood was taken, also with aseptic precautions from the ear the central artery or the marginal vein being used as the case required. In order to obtain accurate comparisons it was considered necessary that the blood and the aqueous should be taken from the same animal. Plasma in preference to serum was employed the osmotic pressure of the two are different, if only by a small amount, and it is the former which comes into equilibrium with the aqueous *in vivo*. It was also considered essential that the blood should be protected from air throughout, since the osmotic pressure varies with the carbon dioxide content; that it should be uncontaminated by anticoagulants at any rate until the corpuscles had been separated off, since in the majority of cases, these substances are known to alter the distribution of its constituents and that throughout all the manipulations it should be kept sterile, since with the activity of micro-organisms a fall in the osmotic pressure of its protein constituents takes place. A sterile needle, connected with a glass tube (*p*), both of which were coated internally with sterile paraffin wax, was therefore inserted into the artery or vein after the ear of the animal had been cleaned and shaved. The tube, supported in a rubber cork, led down to the bottom of a centrifuge tube (*q*), also rendered sterile and paraffin-coated, so that it opened under a layer of liquid paraffin. By sucking a second small tube (*v*), arranged after the manner of a wash-bottle, blood was drawn into the bottom of the centrifuge tube and lay underneath the layer of paraffin without at any time having been in contact with the air. The cork with the two tubes was then withdrawn, and the blood, still under paraffin, was immediately centrifuged. The middle layer of plasma was then pipetted off, and was ready for introduction into the blood cell.

Before use the osmometer was sterilised, the rubber washers and cellophane by autoclaving the glass cells by keeping them in a solution of perchloride of mercury, and drying them with alcohol and ether. The inside of the instrument was then coated with sterile paraffin wax, care being taken to leave the surfaces of the electrodes clear. To compare the conductivity of the aqueous humour before and after the experiment under constant conditions it was necessary to obtain the first measurements after the fluid and the cell containing it had assumed the constant working temperature, and before it had opportunity of coming into association with the diffusible constituents of the plasma. After 0.2 c.c. of the aqueous had been put aside for the determination of its glucose

content and its refractometric value, a sterile vulcanite plug (*s*) was inserted into the open side of the aqueous cell, clamped into place and sealed with paraffin wax. The cell was then filled with aqueous from the syringe, and the tube (*g*), after its lower end had been dipped into sterile liquid paraffin, was pushed home into position so that the aqueous rose a little way up its lumen, its exposed surface being protected from the air by a layer of liquid paraffin, the tube was then sealed into position with paraffin wax. The half-cell was then immersed in the thermostat, and allowed to remain for half an hour until temperature equilibrium had been reached, when the conductivity was measured. Thereafter it was taken out of the thermostat, the clamp (*k*) shutting the outlet of the tube was closed, and, the cell being turned with its inner side uppermost, the plug (*s*) was taken off, the cellophane membrane and its supporting gauze were put into place, the washer being sealed with paraffin wax, and the blood cell (*A*), also paraffin-coated, was clamped into position. Into this the plasma was deposited directly from the centrifuge tube, and a small quantity of heparin added as a precaution, in addition to the paraffin, against subsequent clotting. With the stop-cock open, the tube (*l*) was then pushed home until the plasma surface rose to the mark (*t*), when it was sealed into place with paraffin wax, and the osmometer immersed again in the thermostat. The clamp (*k*) was then opened, and the top of the tube (*l*) was connected with the manometer, whose level was adjusted by the levelling bulb so as to exert a pressure upon the meniscus of plasma approximating that which experience had shown to be the final pressure reading. The stop-cock was then closed. At the end of twenty-four hours the pressure in the air confined between the meniscus and the stop-cock was measured by manipulating the levelling bulb of the manometer until the level of the plasma had reached its original mark as determined by observation through a horizontal microscope. The apparatus was then allowed to stand for two hours to verify the attainment of equilibrium. It was always found that this had been reached within the first few hours. Then, this being the case, the reading on the mercury manometer, corrected for the difference in level of a column of plasma between the meniscus and the cell and for the capillarity of the tube, gave the osmotic pressure of the non-diffusible constituents. The conductivity was then again measured, the osmometer dismantled, and the aqueous was withdrawn for the estimation of its refractive index and its sugar content.

The "sugar" was estimated by the Hagedorn-Jensen method. The refractive index was taken in the thermostat under constant temperature

conditions with a Dipping refractometer (Zeiss) fitted with an auxiliary prism to enable it to deal with one drop of fluid the instrument reads to an accuracy corresponding to  $\pm 37$  units of the fifth decimal place of  $n_D$ . The difference—if any—in the refractive index before and after the experiment demonstrated the efficiency of the membrane in keeping back colloid material. The refractometric method seemed peculiarly appropriate for the purpose, since the power to refract light is a function of the size of the molecules—the property with which we are largely concerned—and is additive, being independent of their chemical nature.

Both arterial and venous blood were used, as also was the normal aqueous humour obtained on first performing a paracentesis upon the eye, and the reconstituted aqueous formed secondarily under the abnormal pressure conditions brought about by the evacuation of the anterior chamber.

The results obtained are tabulated below

I. *Experiments on rabbits using normal aqueous humour*

(a) Colloid osmotic pressure

	No of rabbit	Mano meter reading mm. Hg	Diff in levels mm plasma	Capil larity of tube mm. plasma	Cor rected colloid os pres mm Hg	Mean pres mm Hg	Varia tion mm Hg
With arterial blood	1	20.8	+32	-12	22.3	21.66	+0.64 -1.16
	2	19.2	+30		20.5		
	3	20.5	+35		22.2		
With venous blood	4	21.5	+38	~12	23.5	22.1	+1.4 -1.0
	5	18.8	+30		20.1		
	6	21.0	+35		22.7		

(b) Electrical conductivity of aqueous humour— $\lambda_{18^\circ C} \times 10^5$

	No of rabbit	Conductivity before exp	Conductivity after exp	Difference	Mean difference
With arterial blood	1	1293	1293	0	- 3.3
	2	1313	1306	-7	
	3	1342	1339	-3	
With venous blood	4	1363	1385	+22	+17.4
	5	1300	1328	+28	
	6	1353	1355	+2	

(c) Sugar ' content of aqueous humour grm p c

	No of rabbit	Before exp	After exp	Differ ence	Mean difference	(d) Refractive index Before exp	After exp
With arterial blood	1	0.141	0.141	0	+0.012	1.335168	1.335168
	2	0.138	0.168	+0.030		1.335244	1.335244
	3	0.154	0.159	+0.005		1.335206	1.335206
With venous blood	4	0.170	0.102	-0.068	-0.053	1.335130	1.335130
	5	0.155	0.104	-0.051		1.335206	1.335206
	6	0.164	0.122	-0.042		1.335168	1.335168

content and its refractometric value, a sterile vulcanite plug (*s*) was inserted into the open side of the aqueous cell, clamped into place and sealed with paraffin wax. The cell was then filled with aqueous from the syringe, and the tube (*j*), after its lower end had been dipped into sterile liquid paraffin, was pushed home into position so that the aqueous rose a little way up its lumen, its exposed surface being protected from the air by a layer of liquid paraffin, the tube was then sealed into position with paraffin wax. The half-cell was then immersed in the thermostat, and allowed to remain for half an hour until temperature equilibrium had been reached, when the conductivity was measured. Thereafter it was taken out of the thermostat, the clamp (*k*) shutting the outlet of the tube was closed, and, the cell being turned with its inner side uppermost, the plug (*s*) was taken off, the cellophane membrane and its supporting gauze were put into place, the washer being sealed with paraffin wax, and the blood cell (*A*), also paraffin-coated, was clamped into position. Into this the plasma was deposited directly from the centrifuge tube, and a small quantity of heparin added as a precaution, in addition to the paraffin, against subsequent clotting. With the stop-cock open, the tube (*l*) was then pushed home until the plasma surface rose to the mark (*t*), when it was sealed into place with paraffin wax, and the osmometer immersed again in the thermostat. The clamp (*l*) was then opened, and the top of the tube (*l*) was connected with the manometer, whose level was adjusted by the levelling bulb so as to exert a pressure upon the meniscus of plasma approximating that which experience had shown to be the final pressure reading. The stop-cock was then closed. At the end of twenty-four hours the pressure in the air confined between the meniscus and the stop-cock was measured by manipulating the levelling bulb of the manometer until the level of the plasma had reached its original mark as determined by observation through a horizontal microscope. The apparatus was then allowed to stand for two hours to verify the attainment of equilibrium. It was always found that this had been reached within the first few hours. Then, this being the case, the reading on the mercury manometer, corrected for the difference in level of a column of plasma between the meniscus and the cell and for the capillarity of the tube, gave the osmotic pressure of the non-diffusible constituents. The conductivity was then again measured, the osmometer dismantled, and the aqueous was withdrawn for the estimation of its refractive index and its sugar content.

The "sugar" was estimated by the Hagedorn-Jensen method. The refractive index was taken in the thermostat under constant temperature

conditions with a Dipping refractometer (Zeiss) fitted with an auxiliary prism to enable it to deal with one drop of fluid the instrument reads to an accuracy corresponding to  $\pm 3.7$  units of the fifth decimal place of  $n_D$ . The difference—if any—in the refractive index before and after the experiment demonstrated the efficiency of the membrane in keeping back colloid material. The refractometric method seemed peculiarly appropriate for the purpose, since the power to refract light is a function of the size of the molecules—the property with which we are largely concerned—and is additive, being independent of their chemical nature.

Both arterial and venous blood were used, as also was the normal aqueous humour obtained on first performing a paracentesis upon the eye, and the reconstituted aqueous formed secondarily under the abnormal pressure conditions brought about by the evacuation of the anterior chamber.

The results obtained are tabulated below.

I. *Experiments on rabbits using normal aqueous humour*

(a) Colloid osmotic pressure

	No of rabbit	Manometer reading mm. Hg	Diff. in levels mm. plasma	Capillary of tube mm. plasma	Corrected colloid os. pres mm. Hg	Mean pres mm. Hg	Variation mm. Hg
With arterial blood	1	20.8	+32	-12	22.3	21.66	+0.64 -1.16
	2	19.2	+30		20.5		
	3	20.5	+35		22.2		
With venous blood	4	21.5	-38	-12	23.5	22.1	-1.4 -1.0
	5	18.8	+30		20.1		
	6	21.0	+35		22.7		

(b) Electrical conductivity of aqueous humour— $\lambda_{15^\circ C} \times 10^5$

	No of rabbit	Conductivity before exp.	Conductivity after exp.	Difference	Mean difference
With arterial blood	1	1293	1293	0	-3.3
	2	1313	1306	-7	
	3	1342	1339	-3	
With venous blood	4	1363	1385	+22	+17.4
	5	1300	1328	+28	
	6	1353	1355	-2	

(c) Sugar content of aqueous humour grm. p.c. (d) Refractive index

	No of rabbit	Before exp.	After exp.	Difference	Mean difference	Before exp.	After exp.
With arterial blood	1	0.141	0.141	0	+0.012	1.335168	1.335168
	2	0.138	0.168	-0.030		1.335244	1.335244
	3	0.154	0.159	-0.005		1.335206	1.335206
With venous blood	4	0.170	0.102	-0.068	-0.053	1.335130	1.335130
	5	0.155	0.104	-0.051		1.335206	1.335206
	6	0.164	0.122	-0.042		1.335168	1.335168



As supplementing these figures, a typical estimation of the chloride content of blood and aqueous humour in the normal rabbit is of interest. The estimations were done by Ruszynák's modification of Koranyi's method<sup>(16)</sup>, and the results are expressed as NaCl. This method gives values expressed volumetrically as gram per 100 c c plasma or aqueous, but for the purpose of the present enquiry, in comparing two solutions of so dissimilar molecular aggregation, these results should be corrected to allow for the mass occupied by the molecules of solute, which, owing largely to the presence of large-moleculed proteins in the blood, is very different in the two cases. Thus 100 c c of rabbit's plasma was found to contain 8.6832 gram of solids at 100° C, and the density of the plasma was found to be 1.020. 100 c c of the plasma therefore contains 93.6168 gram water, and since this represents the available solvent, a correction factor of 100/93.6168, or 1.075, gives the true concentration in watery solution. The aqueous on the other hand is practically protein-free; it contains 1.0899 gram solids per 100 c c at 100° C, and its density is 1.007. The corresponding correction factor to be applied is therefore 1.01.

#### NaCl

Arterial plasma	0.603 gram per 100 c c plasma	$\times 1.075 = 0.6482$ gram per 100 gram water
Venous plasma	0.578 gram per 100 c c plasma	$\times 1.075 = 0.6213$ gram per 100 gram water
Aqueous	0.668 gram per 100 c c aqueous	$\times 1.01 = 0.6753$ gram per 100 gram water

Further the "sugar" content of the plasma of rabbit No. 1 was found to be

#### "Sugar"

Arterial plasma	0.136 gram per 100 c c plasma	$\times 1.075 = 0.1461$ gram per 100 gram water
Venous plasma	0.120 gram per 100 c c plasma	$\times 1.075 = 0.1290$ gram per 100 gram water
Aqueous	0.141 gram per 100 c c aqueous	$\times 1.01 = 0.1425$ gram per 100 gram water

From these results it is seen that, owing to the excess of non-diffusible substances in it, the plasma exerts an osmotic pressure of about 20 mm. of mercury greater than the aqueous in the rabbit. The measurements of the conductivity show that when arterial blood was being used there was little or no diffusion of the dissociated ions. The small differences found were almost within the experimental error, but the fact that they all occurred in the same sense seems to indicate that there was a tendency to migration from the aqueous to the plasma. When venous plasma was used, however, there was a definite shift of ions from the plasma into the intra-ocular fluid. It is therefore to be concluded that the total concentration of dissociated salts in the aqueous is practically the same as, but probably a little more than, that required to maintain osmotic equilibrium with the arterial blood, and considerably less than that required in the case of venous blood. These findings confirm, in

the main, those obtained by van der Hoeve(7), who, dialysing aqueous against serum found its conductivity to be the mean between a dialysate of arterial and venous serum. Similarly with the undissociated constituents as represented by the sugar this is present in the aqueous in such a proportion as to be practically in osmotic equilibrium with the arterial plasma. When venous blood was used, the diminished concentration of sugar in the aqueous cell indicated a diffusion into the blood, that is, the initial osmotic concentration of this substance in the aqueous was greater than that in the venous plasma. The refractive index throughout showed no detectable change. Concurrent estimations of the chloride (as the preponderating dissociated constituent) and of the glucose showed that there was more chloride in the arterial than in the venous plasma, and more in the aqueous than either, and that there was more sugar in the arterial than in the venous plasma, while the quantity in the aqueous, when expressed in terms of concentration in watery solution, lay between the two, although nearer that in the arterial plasma than the venous.

II. *Experiments on rabbits using the aqueous humour formed secondarily after paracentesis of the anterior chamber*

This re formed aqueous humour has usually been regarded as having a completely different origin from the normal aqueous. It has long been known to contain more protein than the normal fluid, and has been variously called "albuminous" aqueous, "secondary" aqueous, or "reconstituted" aqueous. The normal aqueous contains all the colloidal constituents of plasma in minute amount (protein, fats, immune bodies, etc.), and the re formed fluid differs from the normal in its constitution only quantitatively by containing relatively more of these difficultly diffusible substances. Further, a fluid of the same chemical composition is formed not only after paracentesis, but under all conditions wherein capillary dilatation occurs, as on radiation of the eye or the application of heat, irritant sub-conjunctival injections, mechanical and chemical irritation of the cornea, the production of venous congestion by subluxation of the eyeball or constriction of the neck, or on the lowering of intra ocular pressure brought about by applying pressure to the eye and suddenly releasing it. Moreover, its formation is prevented under these circumstances by any agency which prevents the capillaries dilating, as stimulation of the sympathetic, the retro-ocular injection of adrenaline, or ligature of the carotid. It would seem therefore that none of these names meets the requirements of the case. In as much as it appears to differ from the normal aqueous only in resembling the plasma more closely I propose to call it the "plasmoid" aqueous.

(a) Colloid osmotic pressure

	No of rabbit	Quantity of aqueous removed on 1st paracentesis c c	Manometer reading mm. Hg	Difference in levels mm. plasma	Capillarity of tube mm. plasma	Corrected os. pres difference mm. Hg
Arterial blood	7	0.1	17.5	-32	-12	19.0
	8	0.25	14.5	+35		16.2
	9	0.32	13.2	+35		14.9
Venous blood	10	0.20	15.5	+30	-12	16.8

(b) Electrical conductivity of aqueous humour  $\lambda_{18^{\circ}\text{C}} \times 10^5$ 

	No of rabbit	Conductivity normal aqueous	Conductivity plasmoid aqueous		Difference in cond. normal - plasmoid aqueous
			Before exp	After exp	
Arterial blood	7	1335	1281	1278	54
	8	1272	1174	1170	98
	9	1365	1217	1217	148
Venous blood	10	1313	1230	1262	77

## (c) Refractive Index

	No of rabbit	Normal aqueous	Plasmoid aqueous		Difference plasmoid - normal aqueous	Approx. protein p c in plasmoid aqueous
			Before exp	After exp		
Arterial blood	7	1 335244	1 337088	1 337088	0 001844	1 0
	8	1 335168	1 339036	1 339036	0 003868	2 0
	9	1 335244	1 339834	1 339834	0 004590	2 5
Venous blood	10	1 335130	1 338428	1 338428	0 003298	1 8

Protein content of normal aqueous of rabbit—0 04 p c

## (d) "Sugar" content of aqueous humour

	No of rabbit	Normal aqueous	Plasmoid aqueous	
			Before	After
Arterial blood	7	0 143	0 148	0 148
	8	0 175	0 175	0 179
	9	0 165	0 172	0 177
Venous blood	10	0 150	0 155	0 111

## (e) Chloride (as NaCl)

Normal aqueous	0 686 grm p c.
Plasmoid aqueous	0 536 grm p c

## III Experiments with different species of animals, using normal aqueous humour and venous plasma

Animal	Corrected osmotic pressure mm. Hg	Approximate protein p c in blood
Rabbit	20 27	5-6
Cat	31 33	7-8
Dog	29 80	7-8
(Man—Deiter (6), using Krogh's modification of Sørensen's apparatus	31 7	7-8)

It is seen that on removing progressively larger quantities of the aqueous humour from the eye, the re-formed fluid, as judged from its refractive index, contains a progressively larger quantity of colloid material. The approximate percentages of protein corresponding with the refractive indices found are given in the table these were calculated by utilising a comparatively large quantity of horse aqueous humour and subtracting the refractive index of a protein-free preparation of this

fluid as obtained by the technique elaborated by Brailsford Robertson<sup>(17)</sup> from its normal refractive index and correlating this value with gravimetric protein estimations carried out on the same fluid. From the results obtained it is seen that the difference in osmotic pressure between the plasmoid aqueous and the plasma due to the non-diffusible constituents is progressively smaller in the same animal as the colloid content of the former increases. Further, it is seen that in the normal aqueous of different species of animals this difference varies with the protein content of the blood. At the same time the measurements of the electrical conductivity and the estimations of the sugar suggest that, in the concentration of their diffusible substances, the plasmoid aqueous bears the same relation to the plasma as the normal aqueous does, that is, it is practically in osmotic equilibrium with the diffusible constituents of arterial blood, being—if anything—in slightly higher concentration than these, while the dissociated salts are in less concentration, and the glucose in greater concentration than that required to maintain equilibrium with venous blood.

#### DISCUSSION

The physiological interest in the comparison of the osmotic pressures of the intra-ocular fluids and the blood lies in the influence which the result must have on any theory of the origin of the aqueous humour. If this latter is a secretion elaborated by an expenditure of energy by the cells of the ciliary epithelium, it remains open for it to have an osmotic pressure greater than, equal to, or less than the plasma. If, however, it is a dialysate from the plasma, when it is equilibrated with the latter, its refraction and its conductivity should remain unchanged. The earlier writers, who held that the aqueous was a transudate and who at the same time found that its osmotic pressure was higher than that of the serum, maintained an impossible position which they apparently failed to recognise. And those who, advocating the same origin and looking upon the intra-ocular fluids as in rapid circulation through the eye, regarded the osmotic pressure of the two as being equal, overlooked the fact that the concentration of negative ions in the aqueous, as shown by the chloride content, is higher than that in the blood.

It is hoped in a future publication to deal with the question of the circulation of the aqueous, but as far as considerations of osmotic pressure go, at the end of each of the experiments detailed above the fluids on either side of the membrane (aqueous and plasma) were in Donnan's equilibrium. During the course of the experiments, when arterial blood

was used, neither the undissociated constituents (as judged by the sugar estimations), nor the ionised constituents (as judged by the conductivity measurements) had altered appreciably in concentration—if anything, they had slightly decreased. Before the experiments commenced, therefore, that is, as these fluids occurred *in vivo*, it follows that their molecular concentration bore the same relationship. When venous blood was used, the smaller concentration of sugar in the aqueous cell at the end of the experiment betrayed a diffusion of this substance into the plasma. The chemical analysis carried out concurrently showed that the sugar content of venous plasma is less than that of arterial plasma; the sugar concentration in the aqueous is seen to be greater than that in the former, and slightly less than that in the latter. At the same time the increased electrical conductivity of the aqueous indicated a diffusion of ionised salts in the opposite direction. As a result of the ionic interchange dependent on the addition of carbon dioxide to the plasma there is an increase of osmotic pressure in the change from the arterial to the venous condition, due to the increase of the bicarbonate content undergoing a greater change in molarity than the concomitant decrease of the chloride content. The concentration of the ionised constituents of the aqueous would therefore appear to be slightly greater than that in the arterial, and considerably less than that in the venous blood. It seems to be the case that capillary blood is more nearly related to the arterial than to the venous blood, a relationship suggested by the findings of Verzář and Keller(18) on the oxygen saturation of "finger blood," and of Foster(19), who found that the sugar content of the latter was practically identical with that of arterial and widely different from that of venous blood. Moreover, in the present case, any remissness in the somewhat exacting technique of keeping the plasma absolutely excluded from the air throughout the complicated manipulations would tend to reduce the arterial plasma to some extent. The deduction that appears to follow is that the molecular concentration of the aqueous is equal to that of a dialysate of capillary blood. Such a suggestion would account, moreover, for the (practical) identity of the concentration of undissociated diffusible substances (sugar) in the two fluids, and the preponderance of cations ( $Cl'$ ) in the aqueous. The osmotic pressure of the aqueous is therefore less than that of the blood, the difference between them being determined in part by the difference in the distribution of their ions, and in part by the difference in the concentration of their colloid constituents.

When the eye is punctured and the normal aqueous is drawn off, the anterior chamber rapidly refills with a fluid containing more colloid than normally. The experimental results detailed above show that

the excess of colloid varies with the amount of fluid originally withdrawn, until on complete evacuation the re-formed fluid closely resembles the plasma. It is said that this plasmoid aqueous is of completely different origin from the normal aqueous, that the latter is a secretion, and that the former is a transudate determined by the altered pressure conditions following the removal of the supporting pressure of the intra-ocular fluids. We find, however, that in all stages of withdrawal, the fluid retains the same molecular concentration relative to the blood as the normal aqueous, that is, osmotically, the two always remain in Donnan's equilibrium. Further, the undissociated diffusible substances (sugar) retain practically the same concentration, the slight tendency to increase of sugar in the plasmoid aqueous is probably within the error introduced by an increase in plasma sugar accompanying the excitement of the experimental manipulation, although, alternatively it might be interpreted as indicating the introduction into the aqueous of "bound" sugar along with the increase of plasma proteins. But the conductivity was found to decrease with the addition of protein to an extent greater than the mere addition of colloid molecules warranted. The correction formula to allow for this inverse relation is (Bugarzky and Tangl<sup>(20)</sup>)

$$\lambda_c = \lambda \frac{100}{100 - 2.5p},$$

where  $\lambda_c$  and  $\lambda$  are the corrected and determined conductivities, and  $p$  the protein p.c. Thus in rabbit No 7, where the measured conductivity of the plasmoid aqueous was found to be  $1281 \times 10^{-5}$  and the protein content 1.0 p.c., the normal aqueous should have had a conductivity of 1307, whereas it was 1335. Corresponding with this, chemical estimation showed a decrease in the concentration of anions ( $\text{Cl}'$ )—a relationship explicable in terms of a system in Donnan's equilibrium. It seems reasonable, therefore, to suggest that the two—the normal and the plasmoid aqueous—may be formed by one and the same process, that on the withdrawal of the supporting pressure of the intra-ocular fluids the capillaries dilate, the amount of dilatation depending on the extent to which the eye was evacuated, the permeability of their walls becomes increased, and the dialysate then formed becomes progressively richer in colloids, while, at the same time, the ionised constituents redistribute themselves to conform with the altered conditions.

If the aqueous and the plasma are in Donnan's equilibrium, not only must their osmotic pressures bear a definite relationship, but a difference in electrical potential must exist between them whose value is given by the formula of Nernst

$$E = \frac{RT}{F} \ln \frac{z}{y},$$

where  $x$  and  $y$  represent the concentration of cations on the two sides of the membrane. Recently, Lehmann and Meesmann<sup>(21)</sup>, using a capillary electrometer and 1/10 N KCl electrodes, one of which was introduced through a cannula into the jugular and the other through a needle into the aqueous, found in cats and dogs that a difference of from 6 to 10 millivolts existed between the two, the aqueous being positive and the blood negative. Moreover, the potential difference between them decreased on equalising their protein contents, either by increasing the protein in the aqueous by performing a paracentesis or by injecting plasma into the eye, or after dilution of the blood proteins (in frogs) by intravenous perfusion with Ringer's solution.

Considering these results in conjunction with those obtained as the values of the vascular pressures in the eye<sup>(1)</sup>, it would appear that the physical forces involved—the difference in the hydrostatic pressure of the aqueous (the intra-ocular pressure) and that in the blood vessels, the difference in the osmotic pressure and in the electrical potential of the two fluids—show nothing inconsistent with the hypothesis that the intra-ocular fluids are formed by dialysation from the capillary plasma, and are in equilibrium with it.

### CONCLUSIONS

(1) Evidence is brought forward which suggests that the aqueous humour is a dialysate of capillary blood, in that, when this fluid is equilibrated with arterial blood, its refraction, conductivity and sugar content remain practically unaltered, any change which does occur being in the direction of equilibrium with venous blood.

(2) The osmotic pressure of the aqueous is the same as that of a dialysate. It is therefore less than that of the blood, the amount depending on the difference in the colloid content of the two fluids. This difference is only a small percentage (about 0.3 p.c. in the rabbit) of the total osmotic pressure (about 6000 mm Hg).

(3) The osmotic pressure of the plasmoid aqueous bears the same relation to the osmotic pressure of the blood as does the normal aqueous. Its molecular concentration is also that of a dialysate, and, as far as the present investigation goes, there is no necessity to ascribe to it a mode of formation differing fundamentally from that of the normal aqueous.

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# THE LOCALISATION OF RECEPTORS INVOLVED IN THE REFLEX REGULATION OF THE HEART RATE

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IN 1859 Marey<sup>(1)</sup> showed that a rise in the arterial blood-pressure was accompanied by a slowing of the heart rate, a fall by a quickening. Since that time numerous investigators have attempted to explain the law of Marey by attributing the change in cardiac rate resultant to change in arterial blood-pressure, either to a central or to a reflex mechanism. That reflex slowing of the heart is brought about by a rise of arterial blood-pressure irrespective of a concomitant change in the cerebral blood-pressure has been conclusively demonstrated by the experiments of Heymans and Ladon<sup>(2)</sup>, Anrep and Segall<sup>(3)</sup>, and Daly and Verney<sup>(4)</sup>. The experiments recorded in this paper were undertaken to localise more exactly the receptors engaged in reflex slowing of the heart.

It has been shown by Eyster and Hooker<sup>(5)</sup> that mechanical distension of the aorta causes a slowing of the heart in the dog. In their experiments the circulation was abnormal in that the superior and inferior venæ cavæ, the ascending and descending aortæ, and the left subclavian artery were occluded. A rise in venous pressure was shown by Bainbridge<sup>(6)</sup> to be accompanied by reflex acceleration of the heart, a result which was confirmed by Sassa and Miyasaki<sup>(7)</sup>, who mechanically distended the auricles and large veins by rubber balloons. As far as we have been able to determine, these experimental results of Eyster and Hooker, and Sassa and Miyasaki, are the only ones published which give direct experimental evidence on the localisation of the receptors engaged in reflex changes in cardiac rate.

## A EXPERIMENTAL RESULTS

Dogs were used in all experiments. Anæsthesia was induced with C and E and fully maintained by an intravenous injection of chloralose, 0.1 grm per kilo body weight.

I *Diminished extra-cardiac pressure* The animals were artificially respired with atmospheric air and the sternum split in order to explore the thoracic viscera. The systemic blood-pressure was taken in the left carotid artery. The pericardium was opened and a Henderson's cardiometer pushed over the ventricles and pressed towards the base of the heart within the pericardium. After tying the free edge of the pericardium around the lip of the cardiometer, the cardiometer itself was fixed rigidly in a clamp, and the outlet tube connected to a mercury or water<sup>1</sup> manometer on the one hand and to a filter pump on the other, for the purpose of recording and of maintaining a negative pressure outside the whole heart. A T-piece fitted with a screw clip was inserted into the tube leading from the pump. This arrangement enabled the negative pressure in the cardiometer to be altered at will.

On lowering the pressure within the cardiometer a slowing of the heart was obtained repeatedly in two experiments. This result was independent of slight changes in blood-pressure in *either* direction (Table I)

TABLE I. Dog 8.0 kilos. 0.8 grm. chloralose. Cardiometer over whole heart.

	Cardiometer pressure mm. Hg	Heart rate per mm.	M.B.P. mm. Hg	
a	0	156	130	
	-2.0	140	120	After 20 secs. duration
	-2.0	140	125	" 75 "
	0	160	120	
b	0	145	118-125	
	-1.5	126	130	After 5 secs. duration
	-1.5	132	102	" 15 "
	-1.5	123	112	" 40 "
	0	135	114	

In a second experiment the subjection of the heart to a negative external pressure of 2 mm. Hg caused immediate cardiac slowing from 144 to 130 beats per minute. This slowing persisted as long as the negative pressure was maintained, viz 32 seconds, and the release of the pressure was followed by an immediate quickening to 145 beats per minute. The mean arterial blood-pressures in the periods immediately before, during, and immediately after the application of the negative pressure were 101, 99 and 102 mm. Hg respectively, and the right auricular pressures 8.0, 7.8 and 7.9 cm H<sub>2</sub>O respectively.

In the majority of experiments the slowing of the heart was not immediate—there was generally a latent period of from 5 to 25 seconds. The cardiac slowing associated with the negative pressure in the cardio-

<sup>1</sup> All cardiometer pressures have been converted to mm. Hg for convenience of comparison.

meter was dependent upon the integrity of the vagi and was found to be independent of the venous pressure taken in the right auricle

II *Diminished extra-ventricular pressure* In this series of experiments the cardiometer, fitted with a rubber membrane and sleeve, was placed over the ventricles only. A reduction in the pressure surrounding the ventricles was followed by slowing of the heart, the slowing again being dependent on the integrity of the vagi. This phenomenon occurred also in the eviscerated preparation. The suprarenals were removed in addition to the stomach and gut, and the liver securely clamped as near the hepatic veins as was compatible with there being no hindrance to the flow of blood through the inferior vena cava. Gross rises in arterial pressure were avoided by placing T cannulae in the descending thoracic aorta and the inferior vena cava near its entrance to the right auricle (after partially defibrinating the animal and injecting 0.1 gm. heparin intravenously) and connecting the side limb of one T cannula to that of the other via a variable shunt resistance. Fig. 1 shows the effect of

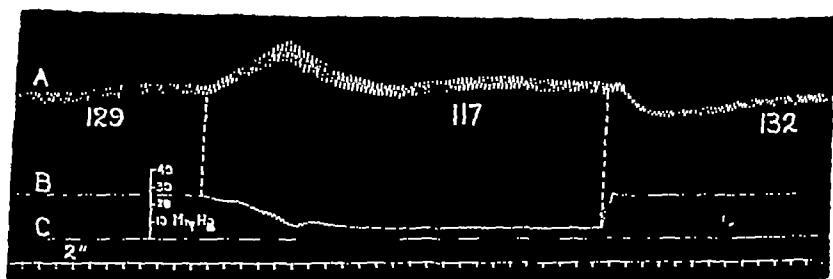


Fig. 1. A = arterial pressure. B = pressure within cardiometer, mm. Hg, the pressure being zero at beginning and end of tracing. C = zero blood pressure. The heart rate is given immediately below the blood pressure trace. Vagi intact. Time = 2 secs.

diminishing the extra-ventricular pressure in such a preparation. The temperature in the inferior vena cava was constant at  $35.5^{\circ}$  throughout. The slowing no longer occurs after cutting the vagi in the neck (Fig. 2).

It was conclusively shown that the presence of the lower portion of the animal was not essential to the manifestation of the reflex cardiac slowing on lowering the extra-ventricular pressure, by sectioning the animal at the level of the eighth thoracic vertebra and connecting the descending aorta via a variable pressure shunt with the inferior vena cava. Fig. 3 is taken from such an experiment after the vagi were cut. The ventricles were again subjected to a negative pressure of 15 mm. Hg.

for 28 seconds, and the heart rates before, during and after this subsection were 126, 132 and 132 beats per minute respectively

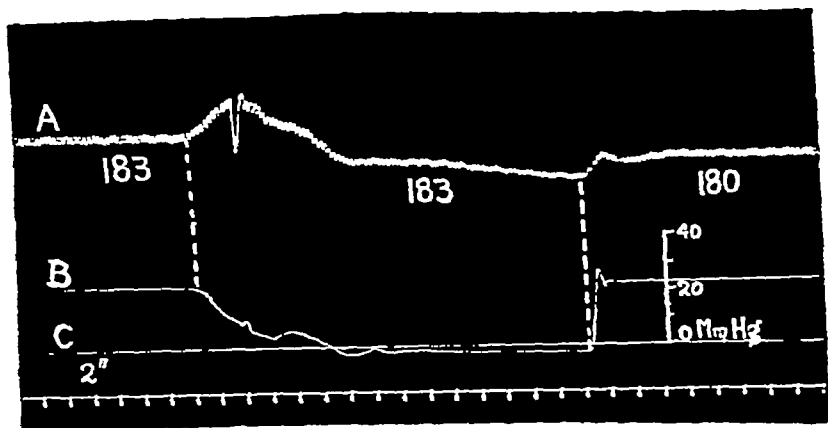


Fig. 2 The letters and figures have the same meaning as in Fig. 1 (q r) Vagi cut.

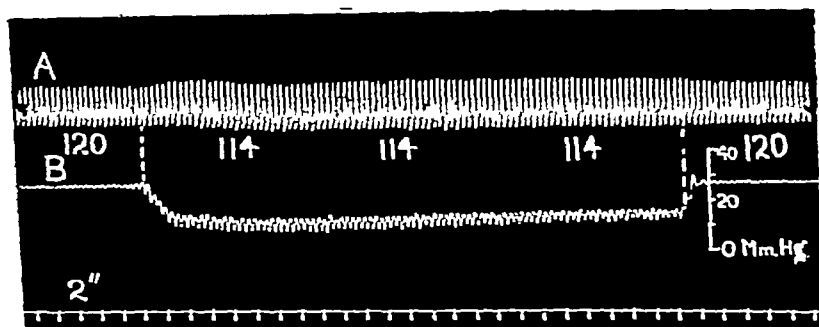


Fig. 3 The letters and figures have the same meaning as in Fig. 1 (q r) Vagi intact.

**III Raised intra-ventricular pressure** In order to produce a rise in left intra-ventricular pressure independently of a concomitant rise in aortic pressure, the following technique was devised. After anaesthetising the animal with C and E and chloralose, artificial respiration was commenced and the chest opened. The subclavian arteries were exposed and two ligatures placed loosely around each. The origins of the aorta and brachiocephalic artery were similarly dealt with, and three further ligatures placed loosely round the descending aorta immediately above the diaphragm. A large T cannula (E, Fig. 4) was then inserted into the

thoracic aorta and tied firmly in position. This cannula communicated by means of a length of wide bore rubber tubing with a T-piece at *H*, the side limb of which passed to a second cannula *D*, which was inserted into the brachiocephalic artery, and directed towards the head. During the actual insertion of this cannula the brain was being supplied with blood by the left vertebral artery only. A third cannula was then tied into the right subclavian artery, and a fourth into the left subclavian artery. The wide tube from the aortic T cannula led to a large cannula which was inserted into the aorta in such a manner that the edge of the cannula just avoided occluding the origins of the coronary arteries, the aorta itself being ligatured immediately distal to the point of insertion of the cannula. During this manipulation the superior and inferior venæ cavæ were occluded, and released immediately the aortic cannula had been tied in position. The final disposition of the cannulae and their connections was as shown diagrammatically in Fig 4, the blood-pressure being recorded from the three sites indicated. In such a preparation, therefore, we are able to record simultaneously the pressures at the aortic orifice (*A*), the cerebral pressure (*B*), and the pressure in the aortic arch (*C*). Further, by impeding the blood flow by compression at the appropriate site, we can vary these pressures independently of each other.

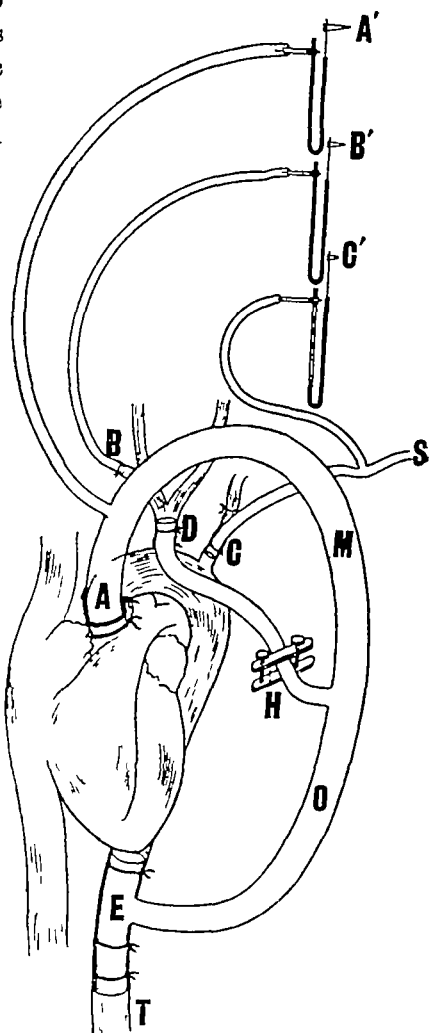


Fig 4 Diagrammatic representation of the disposition of cannulae and tubes used in experiments on the effect, on the heart rate, of independent variations in the blood pressure in the left ventricle, brain and aortic arch

When the aorta was compressed at *T* a rise in all the three pressures was produced, and this rise was accompanied by cardiac slowing. For example, a rise in pressure at the aortic orifice from 118 to 155 mm. Hg, the cerebral and aortic arch pressures undergoing a parallel change, was accompanied by slowing of the heart rate from 133 to 120 beats per minute. On release of the aorta the rate gradually rose, to reach its original value of 133 after an interval of 30 seconds.

Compression at *O* (Fig. 4) raised the pressure at the aortic orifice from 126 to 192 mm. Hg. The cerebral pressure underwent a parallel change, whilst the aortic arch pressure fell from 100 to 14 mm. Hg, the heart rate falling from 156 to 142 beats per minute. Release of the compression at *O* was accompanied by the return of the three pressures to their original values and a gradual increase in the heart rate to 152 beats per minute. This rate was attained 20 seconds after the release.

By compression at *T* accompanied by adjustment of the screw clip *H* so that the cerebral pressure was maintained constant, a slowing was again encountered. For example, the pressure at the aortic orifice rose in one experiment from 100 to 171 mm. Hg, the aortic arch pressure undergoing a parallel rise, the cerebral pressure, however, being maintained at its original level. The heart rate fell from 150 to 145 immediately, to fall still further to 138 after the pressures *A'* and *C'* (Fig. 4) had been maintained at the high level for 30 seconds. 15 seconds after the compression at *T* was released the pressures had returned to their previous levels and the heart rate was then 160 per minute.

Table II shows the effect of compression at *O* accompanied by compensatory tightening of the screw clip so that the cerebral pressure remained constant. It will be seen that a rise in the pressure at the aortic orifice is accompanied by slowing of the heart in spite of the cerebral pressure remaining approximately constant and of the aortic arch pressure falling.

TABLE II.				
Pressure at aortic orifice mm. Hg	Cerebral B.P.	Pressure in aortic arch	Heart rate	
122	108	108	210	
206	102	78	205	Compress <i>O</i> Compensate at <i>H</i>
222	96	55	190	
210	104	62	195	
116	105	105	210	Release <i>O</i> + <i>H</i>

Lastly, compression at *M* produced, as might be expected, a rise in the pressure at the aortic orifice accompanied by a fall in cerebral and

aortic arch pressures In spite of the fall in these pressures a slowing of the heart was produced An example of this result is shown in Fig 5

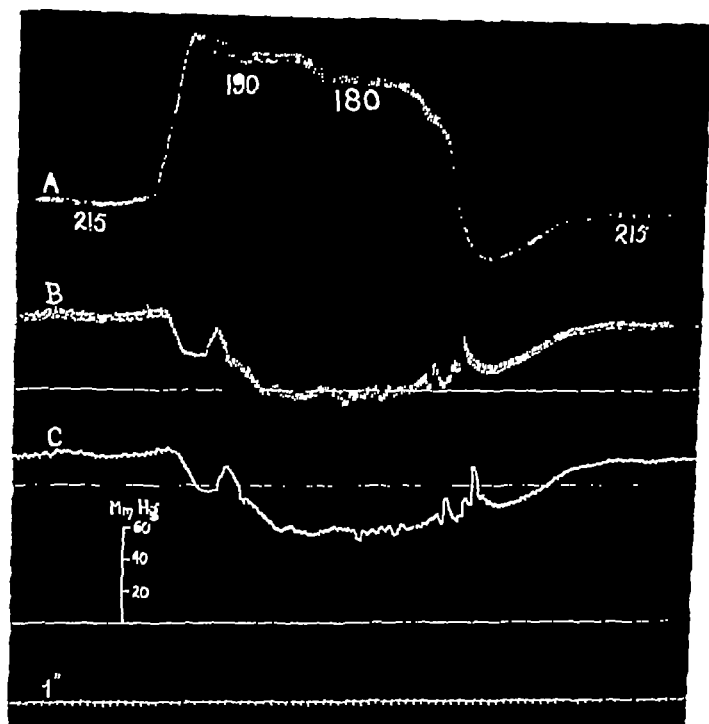


Fig 5 A=blood pressure at aortic orifice B=cerebral blood pressure C=blood pressure in aortic arch The horizontal lines correspond respectively to the zero levels of the three pressures The figures below A give the heart rate per minute

In the experiments illustrated by Table II and Fig 5 a minimal cut only was made in the pericardium, the cut being just sufficient to allow the aortic ligatures to be placed in position In the other experiments of this series the pericardium was freely opened

The position of the aortic cannula was invariably examined post-mortem and it was found that the edge of the cannula was never more than 3 mm away from the origins of the coronary arteries Indeed, in some experiments the distance was 1 mm only (see Fig 6) The distance from the lip of the cannula to the ligature holding it in position (C, Fig 6) was 6 mm The part of the aortic wall central to this ligature, however, rather than that central only to the edge of the cannula, must be considered as being exposed to the changes of pressure registered by the

manometer *A'* (Fig 4) In these experiments, therefore, we have not decisively excluded the potential participation of the most proximal part

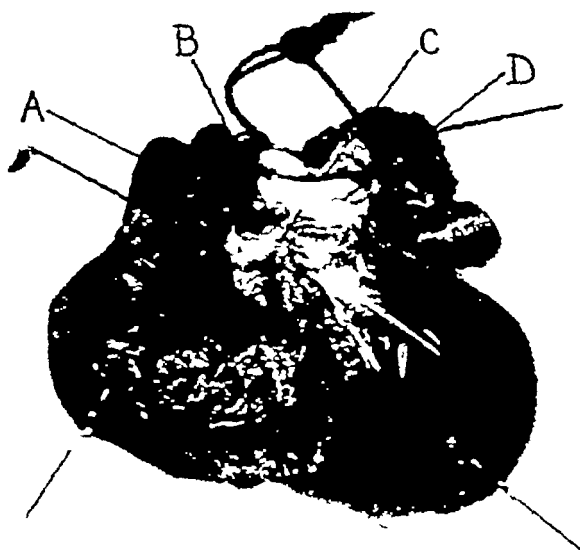


Fig. 6. *A*=aortic orifice. *B*=origin of right coronary artery. *C*=ligature which held aortic cannula in position, showing through the intima. *D*=groove in intima marking the position of the edge of the cannula.

of the aorta in the initiation of the cardio-inhibitory reflex. It is clear, however, that the amount of aortic wall left between the cannula and the ventricle was small, and it seems reasonable to attribute the slowing in rate which accompanied a rise in pressure in the manometer *A'* with constant or lowered pressures in manometers *B'* and *C'* to a rise in the intra-ventricular pressure or coronary arterial pressure or both, rather than to the increased pressure exerted on the remaining fragment of aortic wall.

We may conclude, therefore, from this series of experiments that a rise in left intra-ventricular pressure with a concomitant rise or a fall in aortic pressure, the cerebral pressure remaining constant throughout, or falling, is accompanied by slowing of the heart. The slowing was again found to be dependent on the integrity of the vagi.

IV *Raised aortic pressure* After opening the thorax by splitting the sternum 500–700 c c of the blood was replaced by defibrinated blood obtained from another dog, and 10 mgm. of heparin per kilo body weight



were then injected intravenously. The arterial blood-pressure was taken in the left carotid artery. Two ligatures were placed round the thoracic aorta about one and a half inches apart, the mid-point between the ligatures being from three to four inches below the origin of the left subclavian artery. The aorta was then opened just below the lower ligature and a brass tube 3" in length pushed upwards and tied into the lumen of the aorta so that 2" of its length lay inside. The intra-aortic portion of this brass tube was slightly curved to fit the descending aortic arch and was covered with a thin rubber sleeve tied round it at each end, the extra-aortic portion was connected to a length of rubber tubing fitted with a cannula which was inserted and tied into the distal end of the cut aorta. A fine bore metal tube, piercing the wall of the main brass tube in its extra-aortic course, passed upwards within its lumen and opened on the outer surface in the middle of the intra-aortic portion. By connecting the fine bore tube to a pressure apparatus, the rubber sleeve could be expanded and an internal pressure applied to the walls of the aorta without any alterations in the resistance to the blood flow taking place. During the insertion of the brass tube, the blood supply to the heart was diminished by pulling on ligatures placed loosely round the superior and inferior venæ cavæ, on releasing the ligatures the blood from the descending portion of the aortic arch passed through the brass tube, then through the rubber tube connecting it to the distal part of the descending thoracic aorta. A Cenco blower, with an adjustable pressure valve, supplied the power for the inflation of the rubber sleeve.

Distension of the walls of the aorta by this method was carried out in six experiments. In one experiment the results were negative, in the remaining five slowing of the heart rate occurred without a rise in arterial blood-pressure (Fig 7). In two of these experiments the cardiac slowing was followed by ventricular fibrillation. Garrey<sup>(8)</sup> found that vagal stimulation may stop ventricular fibrillation in the dog, an observation which, when taken in conjunction with our own, suggests that the result on the heart of electrical stimulation of the vagi and of vagal stimulation evoked by aortic distension may be entirely different. In two of our experiments it was found that after vagal section aortic distension was without effect on the heart rate. In the remainder vagal section was not resorted to.

## B DISCUSSION

In this paper we believe we have produced experimental evidence to show that the receptors engaged in reflex cardiac slowing resultant to a

rise in aortic pressure in the dog, are distributed over a considerably wide, area. The experiments described in section IV prove conclusively that some at any rate are situated in the upper portion of the descending thoracic aorta. Others, the presence of which is shown by the experiments recorded in sections I to III, are more difficult to locate precisely, though the evidence points conclusively to their being placed in one or more of

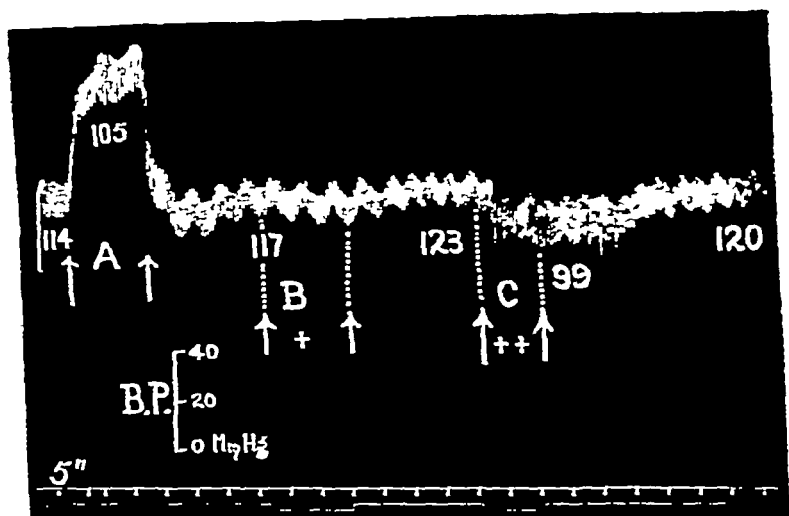


Fig. 7 Between arrows at A rubber connection between brass tube and distal end of aorta compressed at B, aorta moderately distended at C, aorta heavily distended by inflection of rubber sleeve. Heart rate per minute shown by figures below blood pressure tracing. Time 5 secs. Bottom signal, duration of aortic distension.

three situations, *viz* ventricles, coronary arteries and lungs. Further experiments will be required to differentiate satisfactorily between these various possibilities. The point, however, which we wish to stress as the result of the present series of experiments is that we have been able to demonstrate two separate sites in which the receptors are found, namely, the thoracic aorta on the one hand, and the heart or lungs or both, on the other.

### C SUMMARY

- 1 In the dog a negative pressure applied to the whole heart or only to the ventricles causes a reflex slowing of the heart.
- 2 A rise in intra-ventricular pressure with the aortic pressure kept constant causes reflex slowing of the heart.

3 Distension of the aorta without a rise in aortic arterial pressure causes reflex slowing of the heart

4 The slowing of the heart (1, 2, 3, above) is dependent on the integrity of the vagi

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# THE INFLUENCE OF INSULIN UPON THE MAMMALIAN HEART

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College, London )*

WE have investigated the effects of preparations of insulin upon (A) the mechanical condition of the mammalian heart, in the heart-lung preparation and in the whole animal, and (B) the oxygen consumption of the heart in the heart-lung preparation

No previous study has been made of the effects of insulin preparations upon the mechanical behaviour of the mammalian heart under such conditions as would exclude effects upon the rest of the circulatory system as well as upon other tissues of the body. Our attention was drawn to the subject by entirely unexpected observations while injecting insulin into the heart-lung preparation with the sole aim in view of measuring its oxygen consumption

The respiratory metabolism of the isolated heart under insulin has been studied by Burn and Dale(1), who found that there was sometimes an increase, and by Peserico(2) who found a random variation in either direction. The importance of the question of whether or not insulin actually hastens metabolism in active tissues, normal or diabetic, led us to re-investigate the problem with a better control upon the normal rate of metabolism, which the demonstration of the relation between diastolic ventricular volume and oxygen consumption by Starling and Visscher(3) enabled us to maintain

## A. *The influence of insulin preparations upon the mechanical condition of the heart*

In a series of more than twenty experiments we find that adding any one of three commercial brands of insulin to the circulating blood of the "heart-lung preparation" results in a decrease in the volume of the ventricles of the heart. No change was made in the inflow to the heart, in many cases the output was measured and was always found to

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be unchanged. With two brands of insulin (B D H and Boots) there is no change in the heart rate, and in these cases one is forced to conclude that something in the insulin preparation enables the heart to do its work at shorter fibre lengths, and thus more efficiently. With the other brand of insulin (Wellcome) the heart rate is increased, which would tend of itself to decrease the ventricular volume, so it is impossible to argue from experiments with it as to the reason for decreased ventricular volume.

The record of ventricular volume in a typical experiment is given in Fig 1

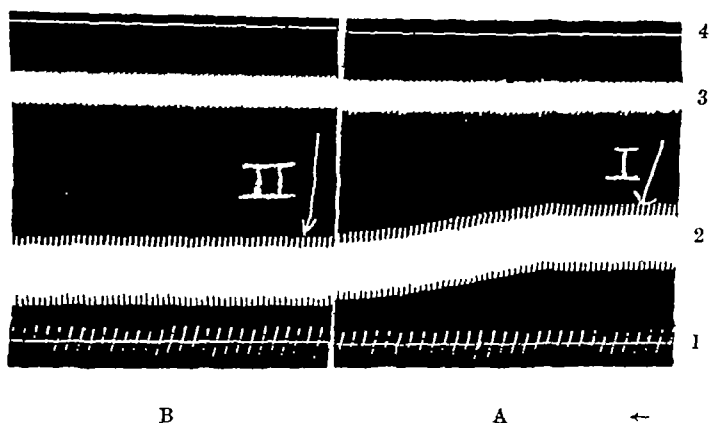


Fig 1 Normal heart-lung showing changes in the diastolic ventricular volume after insulin. 10  $\mu$  of B D H. insulin introduced at points I and II. The second dose is seen to be ineffective. 8 minute interval between A and B. Tracing reads from right to left. (1) Time marks, 10 seconds (2) ventricular volume recorded with Palmer's large piston recorder, down stroke decrease in volume, (3) blood pressure by mercuric manometer 100 mm Hg, (4) horizontal

This effect is noted with hearts from normal as well as diabetic animals, the latter made so by pancreatectomy<sup>1</sup> three days previous to their use in the heart-lung. The effect is neither more nor less pronounced in diabetic hearts than in normal. An example is given in Fig 2.

It has never been possible to obtain any effect from a second addition of insulin to the same heart at any interval after the first. We are unable to suggest any adequate explanation of this peculiarity, but we believe that it indicates that the reaction has to do with the fundamental metabolism. The fact that a second effect cannot be elicited appears

<sup>1</sup> The extirpation of the pancreas was performed by Prof. Starling

to us to prove without reasonable doubt that the action is not due to the addition of alkaline buffers, nor of salt water, nor of small amounts of adrenaline

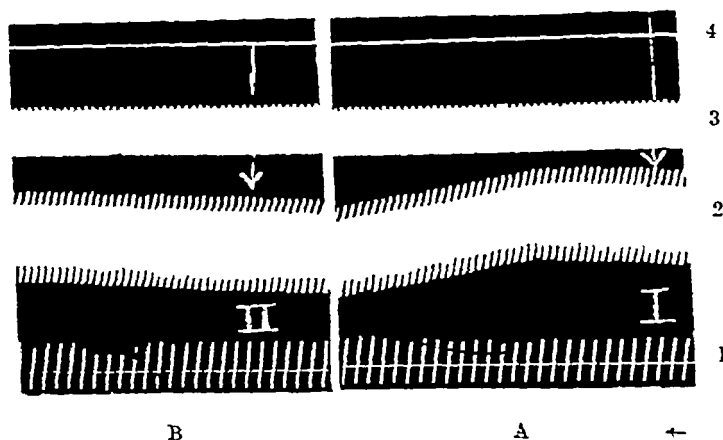


Fig 2. Diabetic heart-lung showing effect of 10 u of B.D.H. insulin introduced at I and II. 15 minutes between A and B (1) Time marks 10 seconds, (2) ventricular volume (3) blood pressure 100 mm. Hg, (4) horizontal.

In the intact anaesthetised animal, administration of insulin results in a rise of arterial pressure of 12–20 mm. Hg, maintained for five or more minutes. There is no change in ventricular volume, but in the intact animal the nervous regulation of the heart and the blood vessels presents a complicated set of conditions which we have not attempted to unravel. For our purpose the important fact is that insulin preparations do affect the intact heart. It is of interest further that one never gets a response from a second injection within fifteen minutes from the first (We have not tried longer intervals in the intact animal). Moreover, the response is exactly the same in normal and in diabetic animals. Figs 3 and 4 present examples.

It is difficult to interpret these findings, but since the response is identical in normal and in diabetic animals one hesitates to ascribe the effect to the "insulin" hormone.

It may be that the heart in the heart-lung preparation—free from any action of the pancreas—is in the same condition as the heart in the diabetic animal. On the other hand, the effect might possibly be due to some impurity present in every brand of insulin we happened to use. We have attempted to decide whether the heart changes we have ob-

served are really due to insulin by inserting a pancreas in the heart-lung preparation, so that we had a heart-lung-pancreas preparation, as described by Babkin and Starling(4)

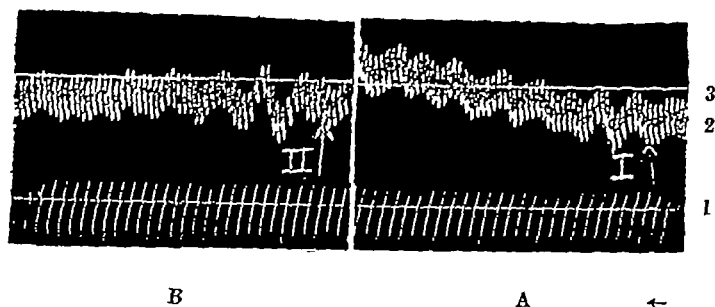


Fig 3 Blood pressure in normal dog showing effect of 10 u of B D H insulin injected in femoral vein at I and II, 20 minutes interval between A and B (1) Time marks 10 seconds, (2) carotid blood pressure by mercury manometer, mean pressure at outset is 125 mm Hg, (3) horizontal

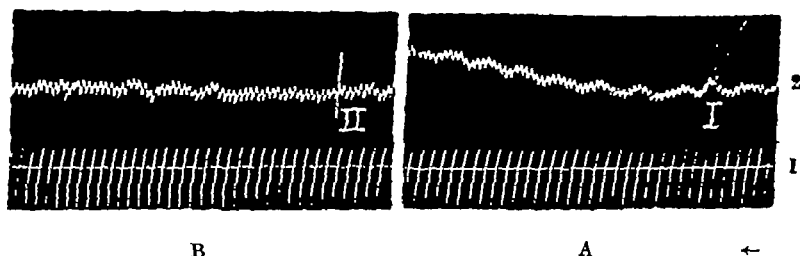


Fig 4. Blood pressure in diabetic dog showing effect of 10 u of B D H insulin injected into femoral vein at I and II, 10 minutes between A and B (1) Time marks 10 seconds, (2) carotid blood pressure, 95 mm Hg at outset.

We did not succeed, however, in obtaining any diminution in heart volume on admitting blood from the pancreas to the heart-lung circulation. The same negative result was obtained when the pancreatic venous outflow was allowed to collect in a Dewar flask, until the venous reservoir was nearly empty, and was then allowed to flow into the heart. The only suggestion that the heart was being supplied with insulin from the pancreas came from the fact that, when insulin was afterwards added, the otherwise invariable effect of a decrease in volume was not obtained, *i.e.* the heart behaved as if it had already received a dose of insulin. It would seem that the blood from the pancreas contained enough

insulin to prevent the effect of a second dose, but not enough, nor in sufficient concentration, to produce a visible change in volume. In order to get a higher concentration in the pancreatic venous blood we perfused the pancreas three times with the same blood before adding it to the venous reservoir, but the blood became very venous and the accumulated  $\text{CO}_2$  interfered with any possible insulin effect. The matter is worthy of further investigation.

*B The influence of insulin preparations upon the oxygen consumption of the isolated heart*

Using the method for oxygen consumption measurement described by Starling and Visscher(3) we find that injection of preparations of insulin which do not alter the heart rate never results in an increased oxygen consumption. In fact, corresponding to the decreased volume of the ventricles there is an actual decrease in the rate of oxygen usage. Table I presents the relevant data. Insulin preparations do not entirely

TABLE I.

Table showing data for the oxygen consumption of the heart-lung preparation before and after the introduction of insulin. This table presents all of those cases in which the heart rate was unaltered and the decreased ventricular volume after insulin was maintained for at least the length of the metabolism observation period. All experiments are with normal hearts except Exp 5, which employed a heart and blood from diabetic dogs. It is to be noted that the oxygen usage always decreases after insulin, concomitant with, and, we believe, because of, the decrease in ventricular volume.

Exp	Dose and brand of insulin	Heart rate beats per min.		Change in average diastolic volume in c.c.	Oxygen consumption c.c./hr	
		Before	After		Before	After
1	15 $\sigma$ Boots	110	116	-3	888	732
2	10 $\sigma$ B.D.H.	160	160	-7	620	485
3	10 $\sigma$ B.D.H.	112	110	-3	255	227
4	5 $\sigma$ B.D.H.	128	128	-3	381	354
5*	5 $\sigma$ B.D.H.	105	100	-1.5	227	204

\* Pancreatectomized animal.

check the slow dilatation of the isolated heart, and occasionally the heart has dilated back to its pre-insulin size before a sufficient period for an accurate metabolism study has elapsed. In such cases, as will be seen in Table II, the average oxygen consumption is practically unaltered. On one occasion the heart dilated very rapidly and the average size for the post-insulin period was 4 c.c. larger than before insulin. In that instance there was, as one would expect, a very large increase in oxygen usage. It becomes apparent that, in all of these experiments in which



TABLE II.

Table showing data for the oxygen consumption of heart-lung before and after insulin, in cases where there was either no significant change, or a rise in average diastolic volume for the period following insulin. It is noteworthy that when the diastolic ventricular volume did not alter appreciably the oxygen consumption remained nearly constant. It is likewise significant that in Exp 4, where the diastolic volume increased rapidly during the period in spite of the temporary lowering due to insulin, the oxygen consumption increased also

Exp	Dose and brand of insulin	Heart rate beats per min		Change in average diastolic volume in c c	Oxygen consumption c c /hr	
		Before	After		Before	After
1	10 $\nu$ B D H.	100	100	+0.5	530	536
2	5 $\nu$ Boots	146	146	-0.5	472	468
3	5 $\nu$ B D H	90	90	-0.5	398	384
4	10 $\nu$ Boots	138	138	+4	524	572

the heart rate is constant, the oxygen usage is in direct proportion to the diastolic ventricular volume, just as Starling and Viisscher(3) found for the heart without insulin

When, however, a preparation of insulin is used which contains an adrenaline-like impurity increasing the heart rate, there is an increase in oxygen consumption, amounting to as much as 60 p c in one instance. This increase occurs in spite of the decreased ventricular volume, which, it should be said, is more transitory with the insulin preparation which shows heart rate effects than with the other preparations. In two of the four experiments in this group, which are presented in Table III, there

TABLE III

Table showing oxygen consumption of the heart-lung following addition of a preparation of insulin causing an increase in the heart rate.

Exp	Dose and brand of insulin	Heart rate beats per min.		Change in average diastolic volume in c.c	Oxygen consumption c c /hr	
		Before	After		Before	After
1	10 $\nu$ Wellcome	120	160	$\pm 0$	396	630
2	10 $\nu$ Wellcome	116	176	-3	352	462
3	10 $\nu$ Wellcome	160	196	+3	485	575
4	10 $\nu$ Wellcome	116	160	+4	352	555

was a very rapid dilatation, so rapid that the average diastolic ventricular volume for the post-insulin period was 3 and 4 c c greater than in the previous period. In these hearts the increased metabolism due to the increased diastolic fibre length is added to the increase due to the higher heart rate, and the total then represents the increase over the pre-insulin rate.

We have, unfortunately, performed only one experiment on the

oxygen consumption after insulin with a diabetic heart In this experiment, shown in Table I, Exp 5, the heart behaved exactly like a normal heart The experiment was entirely satisfactory, but we are unwilling to use it as the basis for an unqualified statement that insulin affects diabetic hearts exactly as it does normal ones, although we believe it to be true

Our results appear to indicate that insulin does not bring about an increase in the rate of oxidation of food-stuffs in the normal heart One preparation we used does indirectly bring about an increase in metabolism by raising the heart rate, but that action cannot be ascribed to insulin, because not all active insulin preparations show it Moreover, the increase in oxygen consumption resulting from its administration is easily accounted for by the heart rate and ventricular volume changes so we feel justified in stating as a conclusion from our experiments that added insulin does not increase the amount of oxidation going on in the isolated mammalian heart It appears justifiable to say further, that our experiments lend no weight to the idea that insulin is a regulator of oxidative metabolism in muscular tissue<sup>1</sup>

#### SUMMARY

1 In the heart-lung preparation commercial insulin causes a more or less transitory increase of the power of the cardiac fibres, which appears as a decrease in the ventricular volume necessary to do a given amount of work.

2 The effect is identical in normal and diabetic hearts

3 In the intact animal, normal or diabetic, a rise in arterial blood pressure follows intravenous insulin injections

4 There is no direct stimulation by insulin of oxidative metabolism in the isolated heart

5 The oxygen usage following insulin is determined by the diastolic ventricular volume of the heart, and the heart rate, exactly as in the heart without insulin.

6 After adding blood perfused through the pancreas to the heart-lung preparation, insulin has no further effect on the heart

The authors wish to record their gratitude to Prof Starling for the great amount of help he has given them both in the experimental work

<sup>1</sup> These results are in conformance with those of Plattner (5) who found that insulin had no influence on the rate of disappearance of sugar from the blood of a heart lung preparation, unless the heart rate was accelerated by the insulin.

and in the evaluation of the results obtained, and likewise for his kindness in providing every facility for conducting this work

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- 2 Peserico *Arch di Fisiol.* 23 p 488 1926
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## THE TWO PHASES OF HEAT PRODUCTION OF NERVE BY R W GERARD<sup>1</sup>

(From the Department of Physiology and Biochemistry,  
University College, London)

IN a previous paper(1) it was shown that medullated nerve (sciatic of *Rana esculenta*) produces more heat during activity than rest, the excess amounting to  $6.9 \times 10^{-5}$  cal per gram per sec of stimulation. An approximate analysis was presented which suggested that there exist two phases—a relatively short and intense one lasting during stimulation, and a less intense one prolonged for nine or more minutes. This analysis, however, was not exact enough to make us certain of the interpretation, and the present study was undertaken to secure more decisive evidence.

### METHOD

The sharpness and accuracy of an analysis increase very rapidly with the quickness of the recording system, so the main objective in the experimental arrangement was speed of response, even when obtained at some sacrifice of stability. The general procedure described previously (preparation of nerve, thermopiles, heat equilibration, shielding of recording system, temperature, etc.) was followed unchanged, but a new galvanometer-relay system was erected.

*Primary galvanometer.* This is of the moving coil type described by Zernicke(2) with very light suspension and manufactured by Messrs Kipp en Zonen of Delft. The particular instrument used, the Zd, has the following characteristics: full period 3 secs, internal resistance  $40\omega$ , and an adjustable magnetic shunt with which the galvanometer can be adjusted to aperiodicity with any external resistance between 60 and  $900\omega$ . As actually employed, with a thermopile of resistance  $250\omega$ , its sensitivity was about  $1 \text{ mm} = 1.3 \times 10^{-9}$  amp at a distance of 1 metre.

This galvanometer, with the same sensitivity as the Ayrton and Mather instrument previously employed, has a period less than half as long, and was adjusted for critical damping, whereas the A and M instrument was much overdamped with the thermopiles used, and though, due to its light suspension, it is somewhat less stable, it proved

<sup>1</sup> National Research Fellow, U.S.A.

quite good enough to stand thermal amplification. It was floated on a bath of mercury, as described in the earlier paper.

*Thermal amplification* A Moll linear thermopile, as previously described, was used, at a distance of about 15 cm. It floated on the same platform on the mercury bath. The beam of light was supplied by a Pointolite lamp running on a set of accumulators and was therefore very constant, so that it was unnecessary to use the surface thermopile to balance the E M F of the linear thermopile in its zero position. The balancing was done by a constant current supplied by a potential divider. The linear thermopile is considerably quicker than the thermal relay, giving practically its full E M F, when a beam of light is suddenly turned on to it, within one second instead of three.

*Secondary galvanometer* Two galvanometers were used at different times in these experiments, an astatic Thompson galvanometer as described by Downing<sup>(3)</sup> and the Zb by Kipp. The Zb is similar to the Zd used as primary, but has an even lighter suspension. The characteristics of this particular one are: full period 2.8 secs, internal resistance  $14\omega$ , sensitivity when adjusted for critical damping at  $25\omega$  external resistance (the resistance of the linear thermopile)  $3.1 \times 10^{-9}$  amp per mm at 1 metre.

With the two Kipp galvanometers and linear thermopile the sensitivity of the entire system (scale at 3.5 metres distance from the secondary galvanometer) was  $1 \text{ mm} = 3 \times 10^{-13}$  amp, and the light showed irregular impulsive movements as great as 30 mm to both sides of the zero position, so that the effective sensitivity for a current of short duration was only about  $10^{-11}$ . These perturbations were much greater than those due to Brownian movements (see below), and it was thought possible that the heating of the mirror and air in the suspension tube of the primary galvanometer by the strong light beam might, by producing air currents, be an important cause of them. For this reason, the sensitivity, which was inconveniently great, was reduced by blocking out about nine-tenths of the beam from the Pointolite lamp, so that as actually used the sensitivity was approximately  $1 \text{ mm} = 2 \times 10^{-12}$  amp. This gave deflections of over 200 mm for a 10 secs stimulation of nerves, and the zero wanderings were much reduced.

With the Zb, or an ordinary slow-moving galvanometer, as secondary, the Zd and linear thermopile showed the usual random disturbances already noted, with a rapid moving-magnet secondary, however, a new type of movement appeared. When the Downing galvanometer was used as secondary and adjusted at, for it, moderate sensitivity and

correspondingly short period (0.8 sec.), it showed astonishingly large and quite regular oscillations. Its speed of response apparently enabled it to follow rapid perturbations of the primary, which were averaged out by the slower instrument. These vibrations, often as much as 50 mm. at 3 metres, were not due to Brownian movements (see, e.g. Einthoven<sup>(4)</sup> and Ising<sup>(5)</sup>), which Prof. Hill has calculated for this system could have attained a maximum root mean square value of only 2 mm. at the sensitivity employed<sup>1</sup>

It was possible, however, largely, though never completely, to eliminate these oscillations by a number of manoeuvres. Making the instrument more sensitive increased its period to 2.5 secs. and so cut out a good share of the disturbances. The entire system was then extremely sensitive (about  $5 \times 10^{-14}$  amp per mm.) and a series resistance of  $500\omega$  was placed in the secondary circuit to reduce this. Advantage was also taken of the possibility of electro-magnetic damping of this instrument (a result of the high magnetic moment of its cobalt steel magnets) and it was shunted by a  $3\omega$  resistance across its terminals. By these means the regular swinging of the spot of light was kept below 5 mm., and the irregular movements were seldom greater. The sensitivity of the system was then  $2$  to  $3 \times 10^{-12}$  amp = 1 mm.

Even at this very high sensitivity, the secondary galvanometer reached a practically maximal deflection in less than 5 secs. (6 secs. for the Zb) after a constant current was thrown into the primary (Fig. 1). This is about six times as rapid as the response of the system previously employed. Careful records were made (and averaged to eliminate random errors) of deflection curves for the secondary galvanometer following (a) a constant current thrown into the secondary, (b) a constant light falling on the linear thermopile, and (c) a constant current thrown into the primary. Analysis of (b) by (a) and of (c) by (b) shows just what lag is contributed by each element of the whole, and in this case the

<sup>1</sup> Ising (5) has calculated for a system similar to the one used here that Brownian movements of the primary galvanometer would limit the useful sensitivity to  $5 \times 10^{-11}$  amp per mm. at 1 metre, if an experimental deflection is to be four times as great as those due to Brownian movement. As he realises, this limit holds only for impulsive or very short currents. Currents of longer duration as in these experiments, even if giving deflections less than the random oscillation, may be readily measured because the Brownian movements, being random and rapid, cancel themselves out to a large extent with a moderately slow secondary galvanometer whereas the steady deflection due to an imposed current is recorded fully. As a matter of fact the disturbances present in this system have been considerably greater than those due solely to Brownian movements, but since they increase less than proportionately to the sensitivity it is practically advantageous to use sensitivities as great as those chosen.

lag is proved to be mainly in the primary galvanometer (This analysis will be further described elsewhere by Hartree and Hill) Since the

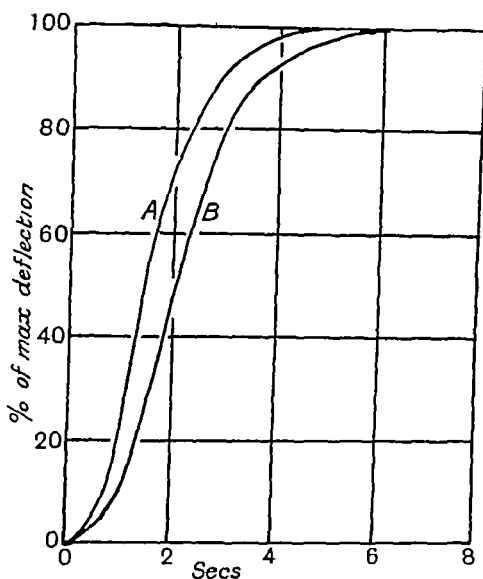


Fig 1 Vertically, deflection of galvanometer in percentage of maximum horizontally, time in seconds. Deflection curves of secondary galvanometer for a constant current thrown into the primary at time zero (A) Downing's galvanometer as secondary, (B) Kipp's Zb galvanometer as secondary

primary must be a moving coil instrument, for the sake of stability, there seems no immediate prospect of further hastening a thermal amplification system

*Method of analysis* A V Hill and Hartree(6) have developed a method of analysing an observed galvanometer deflection, due to experimental heat production, in terms of a control deflection produced by an instantaneous liberation of heat, and so of finding the actual course of the experimental heat. This method, though laborious, is quite exact and has been fully checked in myothermic studies, at first, therefore, it was used boldly in the nerve-heat work. The analyses, however, showed a regular error, so that careful controls were undertaken.

The nerves, lying on the thermopile exactly as during the study of their heat production, were asphyxiated in an atmosphere of nitrogen until no heat was produced on stimulation, and then a very weak current from the induction coil was passed through their length. In

this way a known and uniform liberation of heat was continued for 2, 5, 10 and 15 seconds and deflection curves obtained. If the method of analysis were adequate, these, upon analysis by the instantaneous heating curve, should show the regular heat liberation actually existing, and conversely the ten-second curve should be reconstructable by adding two five-second curves at time 0 and 5 secs, or five two-second curves each starting two seconds after the preceding, etc. Finally, the curve obtained by halving all the ordinates of the ten-second warming curve should coincide with an observed deflection produced by the passage for ten seconds of a current weakened to give a maximal deflection one-half the original. Actually, all these relations are followed with fair accuracy but never quite exactly. Fig 2 shows the observed and built-

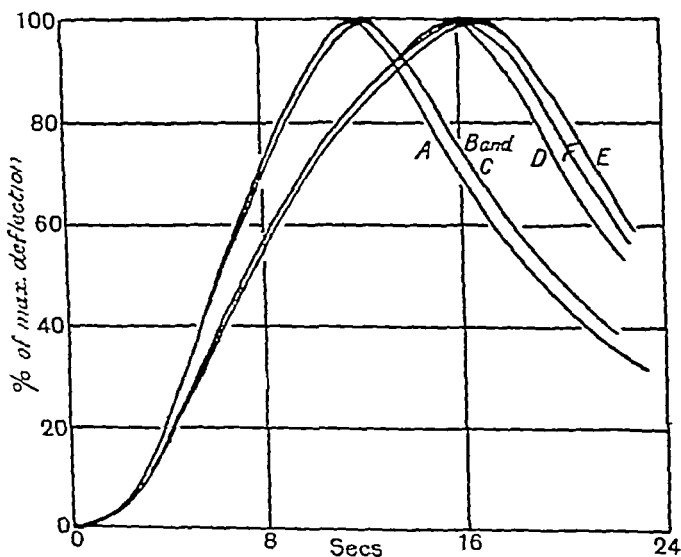


Fig 2. Vertically, deflection of galvanometer in percentage of maximum deflection-horizontally, time in seconds (A) Observed curve for 10 secs. warming through length of nerve (B) Built-up curve for 10 secs. warming by adding 5 observed curves for 2 secs warming each beginning 2 secs after the preceding (C) Observed curve for 10 secs warming with weaker current so that the actual maximum was half as great as for (A) (D) Observed curve for 15 secs warming, (E) Built-up curve for 15 secs warming by adding 3 observed curves for 5 secs warming each beginning 5 secs after the preceding (F) 15 secs warming curve built from  $7\frac{1}{2}$  2 secs curves.

up curves for a 10 secs warming, all reduced to a maximum deflection of 100 and it will be noted that the observed curve tends to descend more rapidly than it theoretically should. Possibly as a corollary of



this, the absolute maximum deflection observed is less than the calculated, sometimes by as much as 25 p c. Similar relations hold for 5 and 15 secs curves. Fig 3 shows a typical analysis of a warming curve. The

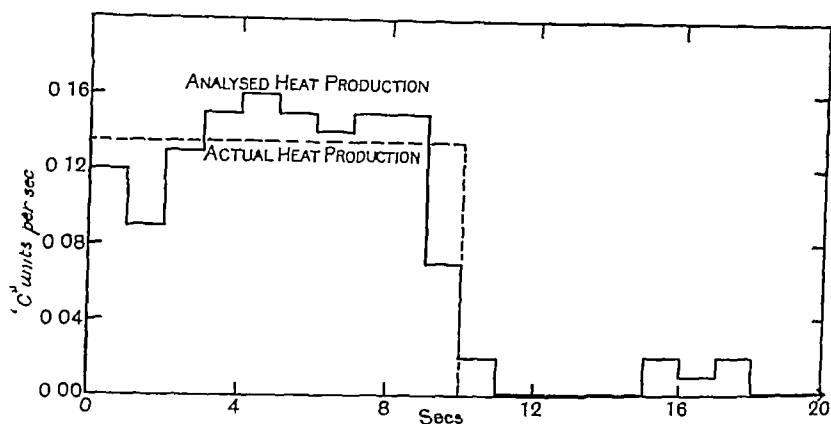


Fig 3 Vertically, quantity of heat per second, in "C" units horizontally, time in seconds. The dotted line represents the block of heat actually liberated, the full line represents the heat given by analysis of the observed galvanometer deflection.

heat was actually liberated in the block indicated by the dotted line, but the analysis shows some lag at the start and a tailing off before the end of the heating, with a small prolongation after the heating is over. It must be admitted, therefore, that under the conditions of these experiments the method of analysis is not rigorous. Theoretically it is applicable to a system, changes in which are governed by linear differential equations with constant coefficients, and that such equations do apply to the development of an E M F in a muscle thermopile, to the deflection of the galvanometers, etc., has been previously demonstrated. The only new factor introduced in the present experiments is the radiation thermopile acting as relay, and it is possible that in this the E M F development follows an equation of a different type. This may explain the observed discrepancies.

The errors so introduced are small and do not conceal the main result of these experiments, but would obscure the exact course of heat production if uncorrected. Fortunately, they can be made negligible by analysing, instead of the whole curve resulting from, say, a 10 secs stimulus of the nerve, the comparatively small difference between this and the curve produced by warming with a uniform current for the same time. The current strength is adjusted so that the maximum deflection is the same as that produced by stimulation. Fig 4 shows

typical stimulus and warming curves for several durations, and it is obvious that the small differences between each pair must give far less

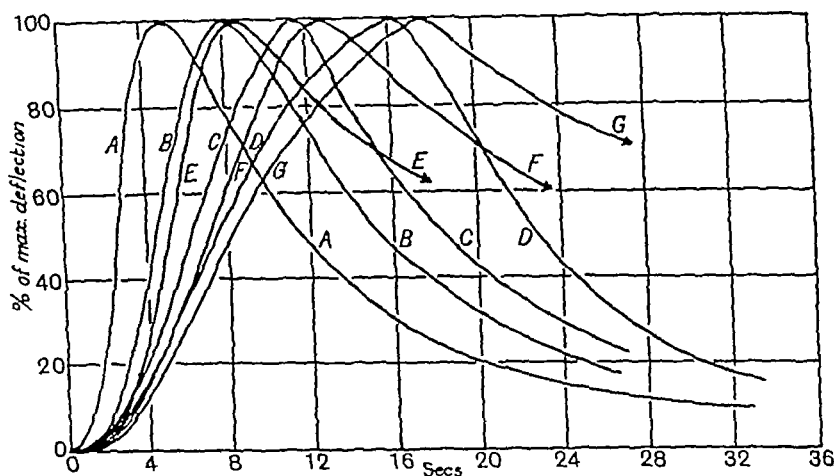


Fig 4. Vertically, deflection of galvanometer in percentage of maximal deflection, horizontally, time in secs. Each curve is drawn through points obtained by averaging a number of observed galvanometer deflection curves, so that individual irregularities have been practically eliminated. (A) "Instantaneous" warming of the asphyxiated nerve by passage through its length of an alternating current for about 0.4 sec. (B), (C), (D) Warming of the nerve in a similar manner but with an even weaker current for 5, 10 and 15 secs respectively. (E), (F), (G) Production of heat by live nerve as a result of stimulation at one end for 5, 10 and 15 secs respectively.

absolute error when analysed than would the entire curve. The result of the analysis of this difference is added algebraically to the block of heat represented by the warming curve, to give the true course of heat production by the living nerve. It may be emphasised that all curves used for analysis are means of several observations made on the same nerves in one day. In this way the random errors resulting from the extreme sensitivity of the system employed were largely eliminated. The curves shown in Fig 4 pass through the actual average points with no smoothing.

## RESULTS

Typical analyses, by instantaneous warming curves employing the method of difference described above, of the heat produced by a nerve as a result of 5, 10 and 15 seconds stimulation are given in Figs 6, 7 and 8. The direct analysis of a 10 seconds stimulation curve without reference to the equivalent warming curve is given in Fig 5 for comparison. This shows a falling off of heat before the end of the stimulus

which was originally interpreted as a sign of fatigue. A 5 secs analysis, however, showed the same effect, and if a nerve exhibited fatigue only

Fig 5

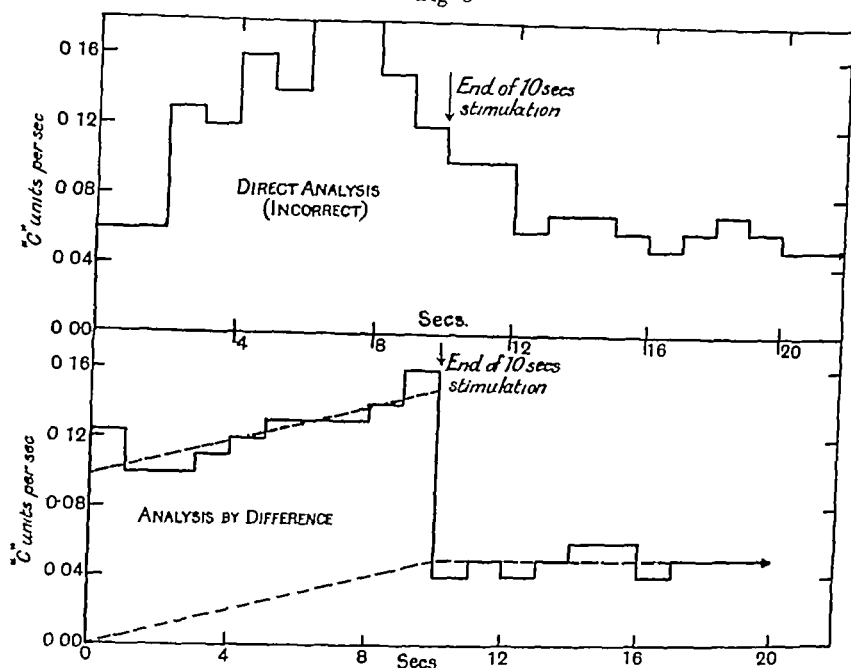


Fig 6

Fig 5 Vertically, quantity of heat per sec in "C" units horizontally time in seconds. Analysis of 10 secs stimulation curve directly by "instantaneous" warming curve, leading to an incorrect result.

Fig 6 Vertically, quantity of heat per sec. in "C" units horizontally time in seconds. Analysis of 10 secs. stimulation curve indirectly, by subtracting the curve for 10 secs warming from the one for 10 secs. stimulation, analysing the negative and positive differences remaining by the "instantaneous" warming curve and adding to this the known heat production corresponding to the 10 secs warming curve. The analysis shows clearly an initial block of heat produced at a constant rate during stimulation, and a delayed heat production reaching a maximum rate by the end of stimulation and then remaining constant during the period represented. The dotted lines indicate these two portions of heat.

at the eighth second of a 10 secs stimulation, it obviously could not show it at the fourth second of a 5 secs stimulation. This led to the further tests described above, and to the method of analysing the difference.

Consistent results were obtained in ten significant experiments. These show clearly the liberation of a relatively large block of heat

during the time of actual stimulation, with a fall at once on its cessation. Continuing from this point there remains in addition a small regular

Fig 7

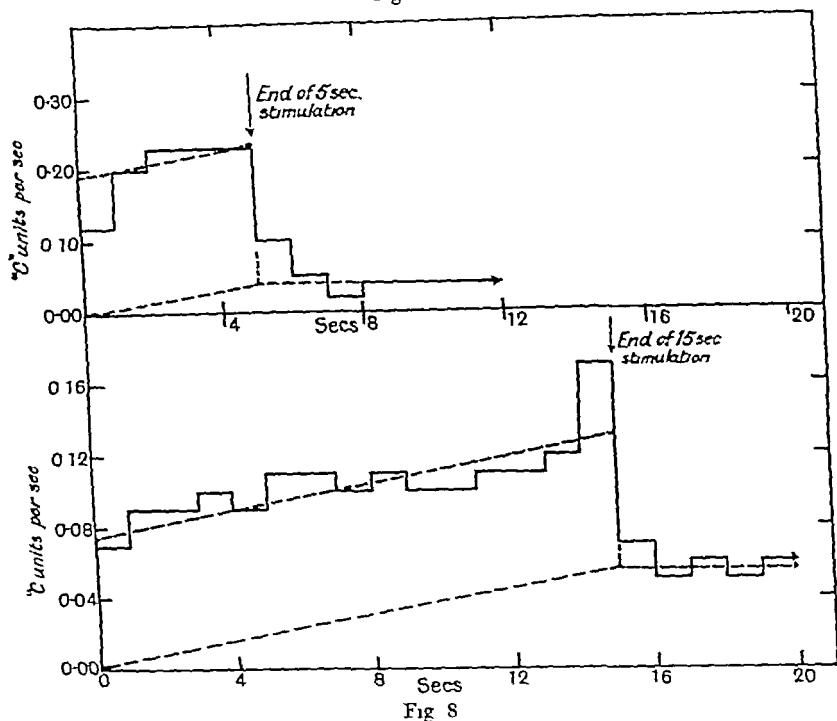


Fig 7 As Fig 6 but for a 5 secs stimulus

Fig 8 As Fig 6 but for a 15 secs. stimulus.

and very prolonged heat production. The dotted lines represent the two portions of heat production—an initial portion lasting at a constant rate during the stimulation, and a delayed portion starting at the beginning of stimulation, reaching a maximum at the end, and then very slowly falling during several minutes to zero. These findings confirm in a general way the interpretation placed upon the approximate analysis already published (1), but in one point lead to another conclusion. There the delayed heat was represented as a curve concave downwards rising to a maximum about 30 seconds after the end of stimulation. These more accurate analyses strongly indicate a break in the "curve" at the end of stimulation, before which time it rises steadily, probably as a straight line, and after which it remains essentially constant within

the period studied<sup>1</sup> If this picture be correct, it follows that the heat liberation for one nerve impulse must be roughly as indicated in Fig 9

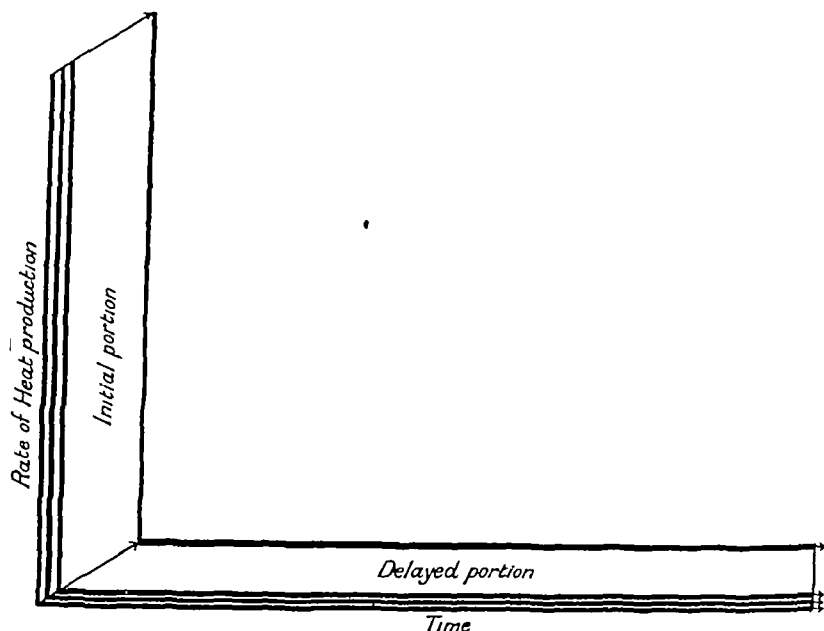


Fig 9 Diagrammatic representation (not to scale) of the heat production due to each nervous impulse, indicating how the observed heat resulting from a tetanic stimulation is built up of these units. The horizontal lines represent the delayed heat, starting at a maximum rate of 0.05 "C" units and slowly falling to zero in about 10 mins. The vertical lines represent the initial heat which probably is largely produced during less than 4σ at a rate 5000 times as intense as that at the start of the delayed phase. The exact form of the initial outburst and of the transition to the delayed heat is unknown and this diagram does not pretend to indicate the true relations of these. No ordinary continuous curve, however representing a single phase, will account for the observations.

The delayed heat, from its beginning, is of a maximum value and, so far as present evidence goes, is a direct continuation of the initial outburst.

*Absolute values* In the previous communication it was shown that the area, and therefore the total heat of a live nerve curve, was 9.1 times the area of an instantaneous warming curve of the same maximum deflection. This relation may be assumed to hold in the present experi-

<sup>1</sup> It is, of course, possible that, just as the slow fall to zero is not apparent in these short time intervals, so also a slow rise might precede it. This would not affect the discussion following.

ments, so if the control curve with the same maximum represents  $C$  units of heat the heat liberated in any time interval may be expressed in terms of  $C$ . For the entire curve, the heat produced is equal to  $9.1 C$ , and since the absolute value for this is known ( $6.9 \times 10^{-5}$  cal per gram of nerve per sec of stimulation(1)),  $C$  can be translated into absolute units.

With the previous galvanometer system, with which the maximum deflection was proportional to the time of stimulus up to 20 seconds, this factor ( $9.1$ ) was independent of the time of stimulus, since  $C$  by definition is proportional to the height of the experimental curve. Using a more rapid system, the deflection is no longer quite proportional to the length of stimulation for the more prolonged stimulations, so that the area of these curves will be rather more (up to 10 p.c. more) than  $9.1 C$ , and must be reduced. The actual values of the initial and delayed portions of heat may be calculated in  $C$  units from such data as is given in Figs 6, 7 and 8.

The average for ten experiments, for 5, 10 and 15 seconds alike, gives  $1.0 C$  unit for the total initial block (i.e. of the part between the parallel broken lines of Figs 6, 7 and 8) and  $0.05 C$  unit per second as the rate during the early part of the delayed portion (at  $15^\circ C$ ). The variation between individual experiments is less than 20 p.c. The initial portion represents, therefore, 11 p.c. of the total heat and in absolute units is  $7.6 \times 10^{-6}$  cal per gram of nerve per second of stimulation. The delayed portion begins at a rate of  $0.05 C$  per sec, absolute value  $3.6 \times 10^{-7}$  cal, and diminishes gradually to zero in a time of about 10 mins. If the rate were constant throughout this 10 mins it would be  $0.02 C$  per sec.

### DISCUSSION

The above results, and especially the numerical values for the two portions of heat, are of especial interest as applied to a single nerve impulse and in a single fibre. It should be noted that during stimulation a nerve fibre is thrown into only intermittent activity so that the average initial heat for a second of activity does not give a fair representation of what is happening during the actual passage of an impulse. Some picture of this may be obtained as follows.

(a) *Heat of a single impulse*. In these experiments, as in the earlier ones, the nerve was stimulated 280 times a second, but it is not possible unconditionally to obtain the total heat per impulse by dividing by this number. Studies on the effect of frequency of stimulation on heat production will be dealt with in a separate communication, and for our

present purpose the fact may be stated that the initial heat per impulse at 280 per second is only 0.26 as much as the heat produced by a single isolated impulse. The assumption may probably be safely made that the delayed heat varies as the initial. The total heat for a single isolated impulse is, therefore, per gram of nerve,  $9.5 \times 10^{-7}$  cal, of which  $1.0 \times 10^{-7}$  cal is the initial portion and  $8.5 \times 10^{-7}$  the delayed portion, and the total heat for an isolated impulse traversing one centimetre length of a single fibre (see (1), p. 248) is  $9.5 \times 10^{-13}$  cal, of which the initial portion is  $1.0 \times 10^{-13}$  cal and the delayed portion is  $8.5 \times 10^{-13}$  cal. The rate of heat production at the start of the delayed period is  $5 \times 10^{-9}$  cal per gram per sec. Under the actual experimental conditions, with 280 shocks per sec, if the initial discharge occupied the full time between successive stimuli, the rate of heat production during this period would be  $2.5 \times 10^{-5}$  cal per gram per sec or 5000 times as intense as the delayed rate<sup>1</sup>.

It is certainly interesting in this connection to note that a very similar ratio of initial rate to delayed rate obtains for muscle heat production under certain conditions. Hartree and Hill(8) have shown for a very short tetanus at 20° C that the maximal rate of heat production during recovery is 0.003 times the total initial heat per second. The initial heat is certainly all liberated within the time of the twitch, which at 20° C occupies about 0.1 sec, so that the rate of initial heat production is ten times the total initial heat, per sec, or 3300 times the maximum recovery rate. Since the relative recovery rate in muscle

<sup>1</sup> The assumption of a duration of about  $4\sigma$  for the initial heat production is certainly not exact, but several facts indicate that it is not many times out. Gasser and Erlanger(7) have shown that at 25° C the great bulk of the action current is passed within  $2\sigma$ , and this same interval is a fair average for the absolutely refractory periods of the various fibres(9), so it might be assumed that the heat is liberated during this time. More direct evidence of the course of the initial heat is obtained from the influence of stimulation frequency on heat production. These experiments have led to the conclusion that the immediate restorative processes in nerve following the conduction of an impulse (as measured by heat) are roughly exponential in character. The numerical values are such that 30 p.c. will be completed in  $4\sigma$  though heat production at a low and diminishing rate must continue beyond this time. If the heat produced during conduction by the discharge of stored energy is only as much as the heat produced during restoration when the energy is being stored, this means that 65 p.c. of the heat will be produced within  $4\sigma$  and the remaining one third in  $50\sigma$  or more. It is probable that the discharge actually represents more than half of the heat and a correspondingly larger fraction is produced within  $4\sigma$ .

It may be well to emphasise that the initial heat production discussed here includes (1) the heat due to actual conduction of an impulse and also (2) that due to immediate restorative (or 'recovery') processes. The prolonged delayed heat appears to be entirely distinct from the latter.

increases with duration of tetanus, for a single twitch it should be lower, and the above ratio higher and so even closer to the value for nerve

It is unwise to press these calculations further, for the exact form of the initial portion of heat liberation and its transition into the delayed portion are not directly measurable, the actual observations giving only the statistical sum of hundreds of impulses for several seconds. A most probable alternate interpretation, however, that the heat is produced continuously and not in two separable phases, for example according to the equation

$$\frac{dH}{dt} = C(e^{-at} - e^{-bt}),$$

where  $C$ ,  $a$  and  $b$  are constants,  $a$  small and  $b$  very large, will not account for the very large amount of delayed heat<sup>1</sup>. It is difficult to avoid the conclusion, therefore, that during and immediately after conducting an impulse the nerve produces heat at a relatively great rate and that following this, as a separable phase, is a very long and feeble heat production

(b) *Relation to chemical mechanism of nerve activity* The existence of a large delayed heat production, occurring as a separate phase, after the nerve has conducted an impulse and restored its machinery for conduction so that it is ready to act again, certainly suggests a "recovery" process. This must consist of the production, removal, transport or change of state of one or more substances. By analogy with muscle, in which a very similar delayed phase appears after the muscle has con-

<sup>1</sup> The argument is roughly as follows

The total heat from time zero to infinity, by integration, is

$$H_0 = -\frac{C}{a} [e^{-at}]_0^\infty + \frac{C}{b} [e^{-bt}]_0^\infty,$$

which becomes  $H_0 = \frac{C}{a} - \frac{C}{b}$ . Similarly the heat produced from any given time  $t$  to infinity is

$$H_t = \frac{C}{a} e^{-at} - \frac{C}{b} e^{-bt}.$$

When  $b$  is very large and  $t$  not very small this becomes  $H_t = \frac{C}{a} e^{-at}$  and  $H_0 = \frac{C}{a}$ . Also for very large values of  $b$  the maximum rate of heat production is

$$\left(\frac{dH}{dt}\right)_{\max} = C \quad \text{and} \quad \left(\frac{dH}{dt}\right)_t = C e^{-at}$$

If  $t$  be taken so that  $\left(\frac{dH}{dt}\right)_t = \frac{1}{500} \left(\frac{dH}{dt}\right)_{\max}$  (which is 10 times as high as the ratio obtained), then  $C e^{-at} = \frac{C}{500}$  so that  $H_t = \frac{C}{a} \frac{1}{500}$ . Since  $H_0 = \frac{C}{a}$ , only 1/500 of the total heat could be produced in the delayed portion. As the experiment shows that 9/10 of the heat is delayed, the postulated continuous single phase is impossible, if an equation of the type given in the text is obeyed.



tracted and relaxed again, it would appear likely that some substance produced during activity is slowly removed, probably by oxidation. Some evidence for this was first adduced by Fillie<sup>(10)</sup> from his findings that (a) a nerve ceased to conduct much sooner in nitrogen than in oxygen free Ringer, and (b) a nerve entirely asphyxiated in nitrogen would regain conductivity for a time when bathed by oxygen free Ringer. The Ringer solution he believed permitted deleterious metabolites, which form only in the absence of oxygen, to diffuse away. Also it appears now quite surely established that an increased oxygen consumption and  $\text{CO}_2$  production accompany nerve activity<sup>(11)</sup>. The relation of oxygen lack to heat production in the two phases, however, does not appear to be the same as in muscle, the recovery heat failing in oxygen lack only as rapidly as the initial<sup>1</sup>. This whole question, as well as the correlated one of nerve fatigue, will be left for treatment in a subsequent paper. A guess as to the actual chemicals involved is also profitless pending further studies, though it is tempting to invoke lactic acid, especially as it is formed so readily in the central nervous system<sup>(12)</sup>.

One clue to the chemical processes of recovery is worth noting. The absolute rate of heat production at the start of the delayed phase is directly proportional to the initial heat, *i.e.* for a 10 secs. stimulus it is twice as great as for a 5 secs. one. This strongly suggests that following conduction some substance is present, in amount proportional to the initial activity, which then enters directly as a single component (mono molecular) into a heat-producing reaction. This is not the case for muscle. Here the maximum recovery rate is not reached for 20–60 secs., which points to a primary reaction of little heat, and also it varies as the square of the initial heat, indicating a bimolecular reaction at the second stage.

#### SUMMARY

1 A thermal-amplification galvanometer system reaching almost full deflection in 5 secs. at a sensitivity of  $1 \text{ mm} = 2 \times 10^{-12} \text{ amp}$  is described.

2 This system does not exactly obey the equations which make

<sup>1</sup> The persistence of delayed heat in the absence of outside oxygen does not finally prove that it is non-oxidative. Without invoking the old "intramolecular oxygen" it is worth noting that hydrogen acceptors, which might make possible anaerobic oxidations, exist in nervous tissue. To consider just one of these, B. E. Holmes (*Biochem. Journ.* 20 p. 872, 1926) has found in the white portion of rabbit brain 52 mgm. of glutathione per 100 gm. of white matter. If this were similarly present in peripheral nerve, were in the oxidised form, and could all be reduced it would permit oxidations equivalent to the total heat produced by nerve in 30 mins. tetanisation.

possible the analysis of galvanometer records, to obtain the time course of heat production. By a method of difference involving a series of control warming curves, however, the error may be made negligible

3 The heat produced by frog sciatic nerves on 5, 10 and 15 secs stimulation was analysed in this manner. The results show clearly an "initial" block of heat produced at a constant rate during stimulation but ceasing at once with it, and also a "delayed" heat, rising to a maximum rate during the period of stimulation and then very slowly falling. The heat production of a single impulse is deduced from this

4 The initial heat constitutes 11 p.c. of the total heat and for one impulse through 1 gram of nerve amounts to  $1.0 \times 10^{-7}$  cal. The equivalent delayed heat is  $8.5 \times 10^{-7}$  cal. The rate of initial heat production is calculated to be about 5000 times the maximum rate of delayed heat production, about the same ratio as obtains in muscle

5 The existence of the delayed heat indicates a "recovery" process separate from the restoration of conductivity during the refractory period. The time course of this heat production suggests that some substance formed during activity is slowly removed, entering as a single component into some reaction.

Finally I take pleasure in recording my indebtedness and thanks to Prof. A. V. Hill for aid and advice throughout this research. I also wish to thank Mr J. L. Parkinson for willing assistance

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## THE STATIC EFFORT AND THE EXCRETION OF URIC ACID By ROBERT CAMPBELL GARRY

*(From the Institute of Physiology, University, Glasgow)*

THE relationship, if such there exist, between type and degree of muscle activity and the excretion of uric acid in the urine forms a very vexed question

The problem, like so many others in metabolism, resolves itself more or less into the general question as to what is to be regarded as a significant degree of deviation of the output of any substance from the mean output. This in turn must of necessity depend on the rigidity of the conditions with which the metabolic experiment is conducted, on the attention paid to the technique of analysis and finally on the accuracy of the method employed.

In this particular problem there is the further complication as to the exact source of the uric acid excreted. Does it arise only as a waste product of muscle metabolism or may it come from other sources such as, for example, synthesis? Further, the conditions governing the actual excretion of the substance may be of importance.

In the first place there seems to be no doubt but that the uric acid content of the blood rises as a result of muscular exercise. Thus Rakestraw<sup>(19)</sup> found that both a short and a long period of exercise brought about an increase of uric acid in the circulating blood at the time of work. In one experiment a further increase was observed after completion of the exercise. Similarly Levine, Gordon and Derick<sup>(13)</sup>, examining the blood of athletes within 30 minutes of completion of a Marathon race, found a decided increase in uric acid. The athlete who won the race showed a smaller increase than the others. The authors conclude that the better the training of the athlete the smaller the increase of uric acid in the blood (cf. Dunlop, Noël Paton, Stockman and Maccadam<sup>(8)</sup>).

Burian<sup>(2)</sup> found an increase in the purine and uric acid content of the fluid used to perfuse the artificially stimulated muscles of a dog, and Macleod<sup>(16)</sup> found that muscle lost "nucleon" nitrogen as a result of exercise.

There would seem, therefore, to be no doubt in the minds of workers in this field that muscular work does influence purine metabolism, but differences of opinion occur as to whether these disturbances of metabolism are reflected in the urine. Thus Lusk(15) concluded that the character of the urine was unchanged after muscular work. Burian observed on himself an increase of uric acid excretion during the hours immediately following muscle activity, but this was compensated for by a subsequent fall, leaving the total excretion for the 24 hours more or less unaltered. More recently Hartmann(10), experimenting on the human subject, noticed a fall in the uric acid and  $P_2O_5$  excretion during the actual hours of work, followed by a compensatory rise leaving the uric acid content of the 24 hours urine unchanged. This fall during work was attributed to diminished kidney excretion during that period. The inference to be drawn is that the post-work rise was due not to any increased formation or liberation of uric acid but simply to the washing out of the uric acid retained during the work period. The experiments of Rakestraw(19) and of Levine, Gordon and Derick(13) suggest however that this explanation may not be the sole explanation of the subsequent rise. It may be further noted that Hartmann started his purine-free diet only on the night before the day of work and ignored the urine secreted on the day following the exercise. His results therefore are not free from objection.

On the other hand Cathcart, Kennaway and Leathes(6) observed an increase in the excretion of uric acid which "follows the exertions and lasts for many hours after them." On examination of their protocols it is seen that this increase appeared on the day of work, usually reaching its maximum on the day following, and it often lasted for at least two days after the actual exercise.

A further study of the effect of voluntary muscular activity upon the output of uric acid and the purine bases was carried out by Kennaway(11)

Dunlop, Noël Paton, Stockman and Maccadam(5) found that, when the subject was in training, exercise caused a fall in uric acid output, whereas, when the subject was not in training, exercise caused a rise in uric acid excretion. This was not correlated with the observed degree of perspiration which is popularly supposed to be an index of physical fitness.

In a recent paper by Cathcart and Burnett(5), where there was no doubt as to the physical fitness of the subject, although the experiments were not directly concerned with uric acid excretion, it is to be

noted that, when the subject was on a purine-free diet, there was a small but definite rise in uric acid excretion during the work period. When the diet was changed to one containing purine no rise took place. Hence it follows that the nature of the diet on which the work is performed may play an important role.

This raises the question as to the normal endogenous source of excreted purine and the influence which diet may have thereon.

Burian and Schur<sup>(3)</sup> differentiated clearly endogenous from exogenous uric acid and considered hypoxanthine of the muscle to be the chief source of endogenous uric acid. Zwarenstein<sup>(22)</sup> has recently shown connection between ingested uric acid and creatinine excretion, thus, in his opinion, strengthening the probability of the derivation of uric acid from muscle. Lewis, Dunn and Doisy<sup>(14)</sup> considered that the increase in uric acid excretion after protein ingestion was due to a general stimulation of all cellular metabolism by the amino-acids. Horbaczewski (cited Burian and Schur<sup>(3)</sup>) saw in the nuclei of destroyed cells, before all in the nuclei of leucocytes, the source of uric acid, going so far as to say that the increase of uric acid in the urine after ingestion of purine-rich food is due to a digestion leucocytosis and subsequent destruction of these cells. It must not be forgotten however that Ackroyd and Hopkins<sup>(1)</sup> have shown that there is a possibility of synthesis of the purine ring in the body from arginine and histidine.

In addition however there is perfectly good evidence that purine-free non-nitrogenous food may quite definitely influence the uric acid excretion.

Thus Cathcart<sup>(4)</sup> showed that on a pure fat diet the uric acid excretion was low in comparison with that on a carbohydrate diet, and he noted that addition of purine-free protein to the diet caused a rise in uric acid excretion. Graham and Poulton<sup>(9)</sup> confirmed these results while Umeda<sup>(20)</sup>, as a result of experimenting on himself and on animals, came to the conclusion that the increased excretion of uric acid on a carbohydrate-rich diet was due to synthesis of uric acid in the body. Macleod and Haskins<sup>(17)</sup>, on the other hand, considered that the excretion of endogenous purines was not affected by considerable variations in purine-free diet.

The possibility of uric acid being "washed out" of the body by large water ingestion is suggested by the work of Cathcart<sup>(4)</sup>. Wilson<sup>(21)</sup>, however, in metabolic experiments where he varied enormously the water ingested, found no constant alteration in uric acid excretion.

Another question of some moment is whether the environment can

play any interfering role Cathcart and Leathes(7) have found that involuntary muscle contraction, in the form of shivering induced by cold, caused a large increase in uric acid output, whereas voluntary muscle exercise, under conditions of warmth, caused a definite fall The etiology of this marked increase in the case of shivering is complicated by the factor of exposure to cold which can by itself, as pointed out by Cathcart, Kennaway and Leathes(8), cause an increased excretion which "outlasts by many hours the exposure to cold"

Since the completion of this work Prof Leathes has called my attention to, and kindly allowed me to refer to, a communication which he and H Orr gave to the Physiological Society in June 1912 In this work they produced evidence to show that increased muscle tension, brought about voluntarily and apart from any reaction to cold, causes a marked rise in uric acid excretion

In view of these findings it was considered that further investigation into the effects of involuntary muscular activity, with the temperature factor eliminated, might provide information of interest

#### *Present investigation*

In the present work the type of muscle activity studied resembled somewhat the involuntary muscle tremor found in the shivering reaction to cold, but any marked temperature alteration was avoided When muscles are maintained in a state of increased tension a fine fibrillary tremor appears in the muscle substance, but, unfortunately, this strain cannot be maintained for more than an hour or an hour and a half at any one time

In the first few experiments this tremor was induced in the arms alone by pulling against fairly powerful springs, but later, tremor was also induced in the leg muscles by maintaining heavy weights in a raised position by means of a foot lever This type of activity may be called static work or static effort The tremor could be felt by the subject of the experiment and also by observers This tremor must not be considered the same as muscle clonus, in which the muscle as a whole contracts and relaxes rhythmically, for the tremor thus induced by continuing strain did not implicate the muscle belly as a whole but seemed to affect individual bundles of muscle fibres in an inco-ordinate fashion The tremor became progressively worse, while from time to time a generalised rigor or "shiver" passed over the entire body Fig 1 is a trace obtained from the subject's arms during such an experiment

Some slight cyanosis was observed in the subject's hands during the experiment. For three or four hours after the experiment the muscles ached dully, there was a tendency to inco-ordination of voluntary movements and the tremor returned on the slightest strain being thrown on the affected muscles. The following day however there was no pain, no tenderness nor stiffness of the muscles.

In one case the course of temperature change was continuously followed by means of a rectal thermometer. At the outset, 3 o'clock in the afternoon, the reading was  $37.1^{\circ}\text{C}$ , at the close of one hour's static work it was  $37.6^{\circ}\text{C}$ .

The diet was purine-free, consisting of 16 oz white bread, 4 oz butter, 3 oz cheese, 2 pints of milk (40 oz) and one apple per diem. The calorie content was more than ample, the subject gaining weight slightly. The water ingestion was not rigidly controlled, but its variation was slight, all alterations in urinary output being explainable by variation in environmental temperature, or by perspiration following on the muscular exertion.

The daily routine was maintained as uniform as possible. The urine was collected in 24 hour samples from 7 a.m. to 7 a.m.

When, as a result of urinary analysis, the subject was judged to be in metabolic equilibrium an experiment was carried out. When the standard level had been restored, without a break in the routine, the experiment was repeated.

The methods employed in urinary analysis were as follows:

Total nitrogen Kjeldahl

Uric acid modification of Hopkins-Folin method

Total purine nitrogen Camerer-Arnstein

Ammonia Folin

Creatinine and creatine Folin

$\text{P}_2\text{O}_5$  simple titration with uranium acetate solution

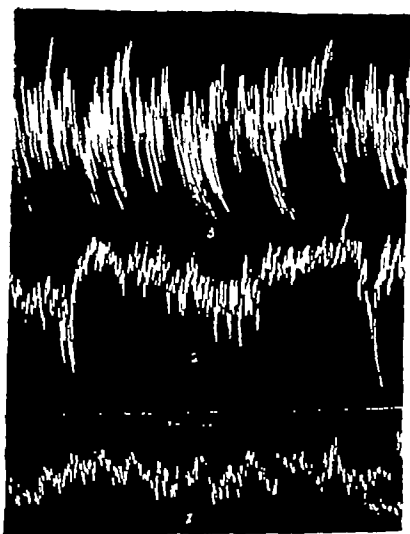


Fig 1 Tremor Time in seconds  
(1) At end of 15 minutes. (2) At end of 30 minutes (3) At end of 50 minutes

In Table I and in Table II two typical protocols are given in detail Table I gives an example of the results which were usually obtained and shows clearly the effect of static work on the output of uric acid and on

TABLE I.

Date	Mean temp ° F	Humidity	Urine volume	Grm. nitrogen in						Crea time	Crea time	Grm. $P_2O_5$
				Uric acid	Other purines	Total N	NH <sub>4</sub> salts	Crea	Crea			
26 XI. 24	49.9	91	860	0.155	0.047	14.00	0.80	0.57	—	—	—	2.67
27 XI. 24	47.4	88	960	0.159	0.055	14.56	0.77	0.60	—	—	—	2.70
*28. XI. 24	42.0	83	830	0.165	0.059	14.20	0.84	0.54	—	—	—	2.70
29 XI. 24	43.0	94	910	0.161	0.060	14.42	0.84	0.53	—	—	—	2.56
30 XI. 24	47.2	83	1040	0.165	0.045	14.80	0.86	0.58	—	—	—	2.70
1 XII. 24	47.5	90	1000	0.148	0.037	14.00	0.83	0.56	—	—	—	2.56

\* Day of static work.

"other purines" Table II is given as an example of a slightly anomalous rate of excretion of uric acid

TABLE II.

Date	Mean temp. ° F	Humidity	Urine volume	Grm. nitrogen in						Crea time	Crea time	Grm. $P_2O_5$
				Uric acid	Other purines	Total N	NH <sub>4</sub> salts	Crea	Crea			
1 XII. 24	47.5	90	1000	0.148	0.037	14.00	0.83	0.56	—	—	—	2.56
2 XII. 24	45.3	88	1015	0.160	0.052	14.32	0.76	0.57	—	—	—	2.84
*3. XII. 24	45.1	84	840	0.154	0.049	14.20	0.78	0.58	trace	—	—	2.27
4. XII. 24	45.5	85	1040	0.181	0.099	14.46	0.79	0.51	—	—	—	2.74
5 XII. 24	45.8	86	970	0.161	0.035	14.84	0.87	0.53	—	—	—	2.70
6 XII. 24	45.6	92	980	0.148	0.052	14.54	0.78	0.59	—	—	—	2.48

\* Day of static work.

In Table II the behaviour of uric acid on the day of static work is worthy of attention. The general rule over the whole series (v Table III) is a slight increase in uric acid excretion on the day of work, but in certain definite cases, where profuse perspiration had accompanied the muscle strain, and where the volume of urine was in consequence much reduced, a slight fall in uric acid excretion was observed. This is not unexpected from the work of Cathcart(4) *vs*. The following day however shows a striking increase in uric acid excretion. Whether this lag in excretion be due wholly to retention in the body as a result of low urinary volume, or due also to delay in formation of uric acid from its precursor, must remain, for the present, a moot point.

For the sake of ease of presentation the results of the twelve different experiments are grouped and averaged in Table III. Each experiment is regarded as covering a six day period, two days before the effort, the day of effort and the three following days.



TABLE III

Average of	Grm nitrogen in					Grm $P_2O_5$
	Uric acid	Other purines	Total N	$NH_4$ salts	Crea tinine	
The days, two days before	0 151	0 040	14 64	0 77	0 52	3 01
The days, one day before	0 152	0 042	14 70	0 77	0 53	3 06
The days of static work	0 158	0 046	14 77	0 78	0 53	2 84
The first days following	0 163	0 045	14 83	0 79	0 53	3 06
The second days following	0 158	0 044	14 64	0 79	0 52	2 94
The third days following	0 153	0 042	14 60	0 80	0 52	2 99

For comparison with these results from the static effort ordinary muscle work was performed under similar conditions of routine and diet. Four such experiments were carried out, walking in the open, walking on a moving platform, performance of work with a bicycle ergometer and with an arm ergometer. Table IV gives one of the protocols and Table V the average of the four experiments.

TABLE IV

Date	Mean temp ° F	Humi- dity	Urine volume	Grm nitrogen in					Crea tine	Grm $P_2O_5$
				Uric acid	Other purines	Total N	$NH_4$ salts	Crea tinine		
2 11. 25	43 6	87	990	0 154	0 023	14 28	0 86	0 55	—	3 45
3 11. 25	45 2	87	1060	0 165	0 029	14 78	0 83	0 55	—	3 48
4 11. 25	43 3	78	1010	0 174	0 036	15 95	0 80	0 59	—	3 83
5 11. 25	44 1	81	1120	0 200	0 037	18 03	0 89	0 65	—	3 49
6 11. 25	36.5	90	1100	0 199	0 034	16 60	0 88	0 61	—	3 35
7 11. 25	40 5	84	1240	0 178	0 037	15 97	0 85	0 51	—	3 39

TABLE V

Average of	Grm nitrogen in					Grm $P_2O_5$
	Uric acid	Other purines	Total N	$NH_4$ salts	Crea tinine	
The days, two days before the exercise	0 158	0 026	14 88	0 76	0 57	3 41
The days, one day before the exercise	0 160	0 032	15.12	0 80	0 56	3 37
The days of exercise	0 164	0 033	15 77	0 78	0 62	3 39
The first days following the exercise	0 178	0 035	16 36	0 87	0 64	3 37
The second days following the exercise	0 169	0 031	15 71	0 84	0 61	3 24
The third days following the exercise	0 169	0 032	15 01	0 83	0 55	3 38

### *Discussion of the results*

In connection with the static effort there is a definite, although small, rise in uric acid output, an increase persisting for 48 hours after the day of experimentation. This was invariably the case although the results varied in degree.

The excretion of other purine nitrogen follows the alteration in the output of uric acid without itself showing much change.

Similarly, in ordinary muscle activity, there is an increase in uric

acid excretion but here the increase is somewhat greater. This increase agrees with the findings of Cathcart, Kennaway and Leathes<sup>(6)</sup>

As far as the present results go there is no doubt that muscle activity, be it voluntary or "involuntary" as in the static effort, causes an increase in uric acid excretion.

The work of Dunlop, Noël Paton, Stockman and Maccadam<sup>(8)</sup>, of Kennaway<sup>(11)</sup>, and of Levine, Gordon and Derick<sup>(13)</sup> suggests that physical "fitness" may alter the degree of increase in excretion of uric acid in response to muscle activity, this possibly partly explaining the contradictory results to be found in the literature of the subject.

In the same way the question of temperature is pertinent. The assessment of the degree of influence which temperature may exert is, however, a very difficult matter. In the laboratory, carrying out routine analysis, the subject is theoretically, although rarely in practice, exposed to a uniform temperature, but once outside the laboratory he is exposed to all the capricious thermal moods of our changeable climate. Cold, especially dry cold, acts as a general metabolic stimulant, and, in this general "tonic" action Cathcart, Kennaway and Leathes see the source of the increased uric acid excretion which results on exposure to cold.

At one period of the research there was a short spell of frost and snow. During that period the excretion of uric acid rose far above the usual level. This alteration was coterminous with the period of cold and the rise was well beyond the limit of experimental or day to day variation.

As regards the other urinary constituents there is little worthy of note. Nitrogen excretion is increased mainly on the day of work and on the day following, this being less obvious in the static effort group.

The alteration in ammonia excretion is not significant.

The behaviour of creatinine is peculiar. In the well-known work of Pekelharing<sup>(15)</sup>, increased muscle tone led to an increased excretion of creatinine, an increase not found after normal muscular exertion. Leathes and Orr<sup>(12)</sup> confirmed this, though the increased output of creatinine was much less than that of uric acid.

In the present series however the static effort shows little effect on creatinine excretion while ordinary muscular work very evidently does so.

A trace of creatine was detected but twice as a result of static effort, both times in the urine of the work period, in view of the difficulties of colorimetric estimation, however, all the results in connection with creatinine and creatine are regarded with some little distrust.

At odd times lactic acid was looked for in the urine of the days of static work Hopkins' thiophene test was used and was always negative

The excretion of  $P_2O_5$  remained constant during muscular work, but on the days of static effort it showed a decided fall

### CONCLUSIONS

(1) Static effort of the type employed, as likewise ordinary muscular exertion, increases the excretion of uric acid on the day of work, the increase extending into the two following days

(2) The output of total nitrogen is similarly affected

My most grateful thanks are due to Prof Cathcart for the suggestion of this work and for his advice and interest during its progress

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FCETAL BLOOD-GAS TENSIONS AND GAS TRANS-  
FUSION THROUGH THE PLACENTA OF THE  
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It has long been known that the developing animal absorbs oxygen and gives off carbon dioxide. In 1674 Mayow<sup>(1)</sup> showed that the egg dies if the shell is varnished. He suggested that in mammals the placenta is the route by which these gases pass to and from the developing foetus and that in fact it functions as a foetal lung. About 1700 Hulse and Ray<sup>(2)</sup> adopted and extended the same opinion. In 1798 Schehl<sup>(3)</sup> observed that in the umbilical vein the blood is red and in the umbilical artery it is blue. In 1876 Zweifel<sup>(4)</sup> showed spectroscopically that oxyhaemoglobin existed in the umbilical cord. In 1877 Zuntz<sup>(5)</sup> demonstrated that in rabbits with maternal asphyxia the mother and foetus die at the same time, but that if the cord be clamped at the moment of asphyxia the foetus dies 10-20 minutes after the mother. This suggested that by not yielding up its oxygen to the mother it lived longer. He also stated that during maternal asphyxia the colours of the bloods were reversed, the blood in the umbilical artery being brighter than that in the vein. From this he deduced that the placenta removed oxygen from the foetal blood and passed it on to the mother during maternal asphyxia. In 1884 Cohnstein and Zuntz<sup>(6)</sup> investigated experimentally the mechanism of the gaseous interchanges between the mother and the foetus, using a number of different mammals, of which the pregnant sheep was the most important. They confirmed numerically the observations of Schehl that the bright red blood leaving the placenta contained more oxygen and less carbon dioxide than that going to the placenta. From 1888 to 1893 Charpentier and Butte<sup>(7, 8)</sup> published a series of papers pointing out from clinical experiences supported by experiments on rabbits, that after a big maternal haemorrhage the colour of the blood in the umbilical artery was brighter than in the umbilical vein. They suggested that the foetal tissues were yielding up oxygen to

supply the mother, that is, it was a case of reversal of oxygen passage across the placenta. In 1909 Bohr(9) discussed the question in Nagel's *Handbuch*. He pointed out that possibly oxygen and carbon dioxide diffused across the placenta, but before the hypothesis of secretion could be rejected certain facts had to be considered. The lung and the placenta are not analogous in regard to structure. Besides having two series of blood vessels or sinuses the placenta has separating the maternal and foetal bloods a layer of active cells, the trophoblast, and a layer of syncytial protoplasm. Further, it has a very active metabolism, and a number of intermediate processes were known to occur involving absorption of oxygen and excretion of carbon dioxide. In fact it was in his opinion a moot point as to which was the actual mechanism.

In regard to the passage of gases other than the normal blood-gases much work has been carried out in France. Nicloux(10,11,12), working alone and in collaboration with Balthazard, has investigated very carefully the passage of carbon monoxide. He concludes that it diffuses across provided that sufficient time elapses for the slow dissociation of the carboxy-haemoglobin to create an adequate pressure gradient in the plasma at the placental membrane. It then appears in the foetal blood. If, however, the dosage of carbon monoxide is too great the dissociation does not occur before the maternal death and so the gas does not diffuse across. The foetus dies of pure asphyxia without having any carboxy-haemoglobin in its blood.

So far the only measurements made of the foetal blood-gases have been the percentages in the arterial and venous bloods of the umbilical cord. The quantities in the mixed blood of the carotid artery going to the brain have not been measured.

If diffusion is taking place the partial pressures of the gases on opposite sides of the placental membrane should be different and such as to allow for diffusion. Neither Bohr nor Cohnstein and Zuntz ever measured the gas tensions in the foetal bloods, still less compared them with those in the maternal blood.

In this paper the problem of the mechanism of placental gas trans- fusion has been approached from three points of view.

(a) The measurement of the absolute partial pressures of the blood-gases in the mother and foetus

(b) The measurement by differential tonometry of the relative tensions in the maternal and foetal bloods at the same moment

(c) Reversal of gas current

### A *Measurement of fetal and maternal blood-gas tensions*

The determination of the partial pressures was carried out by constructing dissociation curves and obtaining the gas tensions from the curves by measuring the gas contents of the bloods

The animals used were goats. This animal was selected first because instead of a litter it has twins, occasionally a single foetus. These foetuses weigh about 1.2–1.5 kg and so are sufficiently big to be operated on without difficulty. Rabbits and cats have litters and the young are too small for accurate quantitative work. In the second place the goat is not grossly expensive. The sheep (used by Cohnstein) has the necessary requirements but is three to five times the price of a goat.

The gas contents were measured by means of Van Slyke's constant pressure blood-gas pump. This was used in preference to the Barcroft differential manometer or the Haldane blood-gas apparatus because the young cells being active were found to self-reduce before accurate measurement could be made. The pump was quicker. Also it was possible to obtain figures for all three gases on the same sample of blood.

The dissociation curves for oxygen and carbon dioxide were made by Barcroft's method using Barcroft saturators. The gas volume in the saturator was about 220–250 c.c., the blood volume was 5 c.c. or less according to desire, the time of saturation was fifteen minutes. It was found that equilibrium was attained in this period. The bloods after saturation were run off into tubes under liquid paraffin and the gas contents determined. The gas mixture left in the saturator was analysed by the Haldane apparatus, the total pressure being taken with a small manometer in the bath before running off the blood. In determining the curves each point was determined separately. This lent to greater accuracy than if all the points were determined together, six bloods being equilibrated simultaneously and then six gas determinations being carried out.

The maternal goat was at or near full term anaesthetised with urethane 1.0 to 1.5 grm per kg subcutaneously and 3–4 hours later induction with ether was performed. The quantity required was negligible and usually only an occasional whiff of ether was necessary to keep the animal under anaesthesia thereafter, very often none at all.

The breasts were amputated, since they are hypogastric in position in the goat. A cannula was put in the trachea and respiration carried on through that. The whole goat was then immersed in a domestic bath containing saline at 38° C. The abdomen was opened in the mid-

line and the two horns of the uterus exposed. One horn was incised over a bloodless area and, the membranes being cut through, the foetus was delivered into the bath of saline with as little manipulation as possible and with its snout kept constantly below the saline. In this way the conditions approximated to those *in utero* but allowed of experimental observations being carried out. The placenta was left intact.

The uterus did not retract, probably because of the anæsthesia and because the placenta, which in the goat covers the major portion of the interior, was not removed. Retraction set in when it was removed.

The bloods were obtained by inserting cannulæ into branches of the umbilical vein and artery for the arterial and venous bloods and into the foetal carotid artery for the blood going to the brain. Maternal arterial blood was obtained from the carotid artery and venous blood from the uterine vein when required.

*Measurement of percentages of gases in foetal blood.* The samples of blood taken all came from foetuses at delivery from the uterus, the placentæ in all cases being intact. The foetus had made no respiratory movements, and was kept entirely submerged in the bath at 38° C. The bloods taken were analysed at once in the Van Slyke apparatus. Table I shows the figures obtained together with the corresponding figures obtained by Cohnstein and Zuntz.

TABLE I. Foetal blood gas contents per 100 vols of blood (in percentages)

Goat	Oxygen			Carbon dioxide		
	F.A.B	F.M.B	F.V.B	F.A.B	F.M.B	F.V.B
B	7.0	6.0	6.0	28.7	31.0	35.0
C	8.5	4.2	0.25	32.0	40.0	45.2
D	5.0	4.5	0.9	35.0	37.0	41.8
M	12.0	9.0	4.0	24.0	34.7	37.6
N	9.7	4.7	3.0	26.0	30.8	34.8
O	7.5	7.0	3.5	34.0	45.8	54.8
Average	7.96	5.9	2.94	29.9	36.0	41.6
Cohnstein and Zuntz	6.3	—	2.3	40.5	—	47.0

F.A.B = Foetal arterial blood from Umbilical V.

F.M.B = Foetal mixed blood to brain.

F.V.B = Foetal venous blood from Umbilical A.

The average total oxygen capacity was 17.4 c.c. oxygen per 100 c.c. of blood. This gives an average percentage oxygen saturation of 45.0 p.c. in foetal arterial blood, 33.1 p.c. in foetal mixed blood and 16.6 p.c. foetal venous blood.

*Measurement of gas tensions in foetal blood.* The indirect methods

described above based on dissociation curves and blood-gas contents were used.

Table II gives the analyses of equilibrations at different gas tensions on a number of bloods from a series of foetal goats. For brevity the whole series of points is grouped in one table giving the percentage oxygen saturation at known oxygen and carbon dioxide tensions, together with the carbon dioxide content per 100 vols of blood. It is the basis of the curves in Figs 1 and 2.

TABLE II. Oxygen saturation and carbon dioxide percentage of foetal blood at varying tensions of oxygen and carbon dioxide.

Goat	Tension of gases in saturator		Volume of carbon dioxide per 100 vols blood c.c. per 100 c.c.	Total oxygen capacity c.c.	Percentage oxygen saturation p.c.
	CO <sub>2</sub>	O <sub>2</sub>			
H	73.6	0.91	50	19	15.8
H	40.9	1.14	32	19	15.8
O	28.7	10.38	23	15.5	12.9
O	17.9	10.88	19	15.5	12.9
O	50.3	11.4	38	15.5	16.0
O	57.5	12.7	38	15.5	16.1
O	38.6	14.7	27.5	15.5	19.3
D	23.8	15.7	23	18	22.2
O	61.2	16.7	41	15.5	16.1
D	15.3	18.2	9	18	30.6
M	40.2	23.7	29	19	29.2
T	50.5	30.0	32.5	17	38.5
D	26.8	31.5	20	18	36.1
T	68.3	32.7	41.5	17	41.5
T	21.0	35.6	15	17	50.0
D	29.1	40.0	21	18	50.0
D	22.4	42.4	18	18	55.5
M	40.3	42.8	28	19	52.7
B	5.04	48.8	10	19	63.3
O	63.2	56.7	35	15.5	61.3
M	24.5	63.8	15	19	76.4
D	17.9	69.4	12	18	75.0
T	72.1	73.0	40	17	76.4
T	10.3	80.0	11	17	85.4
D	17.6	88.0	12	18	88.8
D	26.5	89.0	17	18	89.0
O	71.0	93.8	38	15.5	85.7
O	64.0	98.0	33	15.5	90.3
D	21.5	102.0	15.5	18	91.7
D	32.0	104.6	19	19	92.1
B	25.6	107.0	18	19	94.7
B	38.7	110.2	20	19	94.7
T	50.0	110.0	28.5	17	91.2
H	73.6	121.0	37.5	19	79.5
H	31.5	122.0	20.5	19	97.4
C	39.7	122.0	21	15.5	96.0
B	60.5	134.7	30	19	100.0
B	26.1	144.6	15	19	100.0

The dissociation curves of these animals, while not coinciding, agreed sufficiently closely for an average curve to be drawn through all the



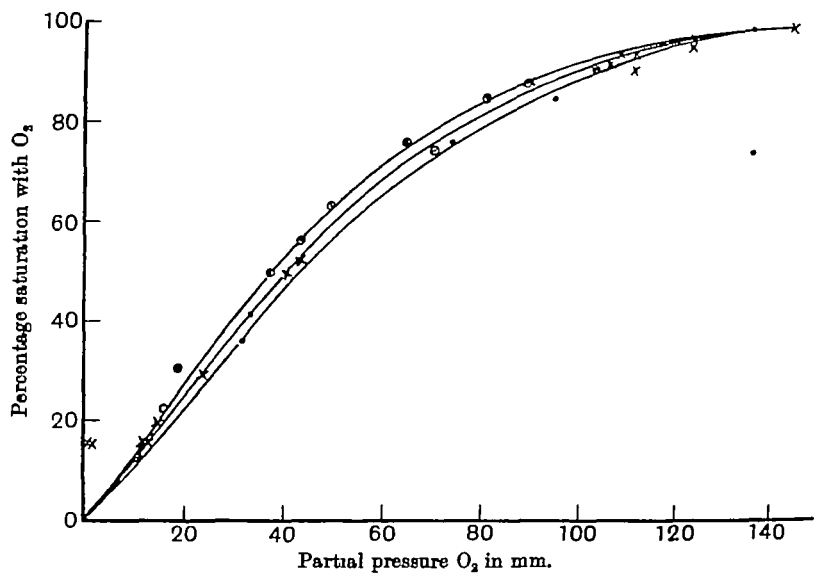


Fig 1

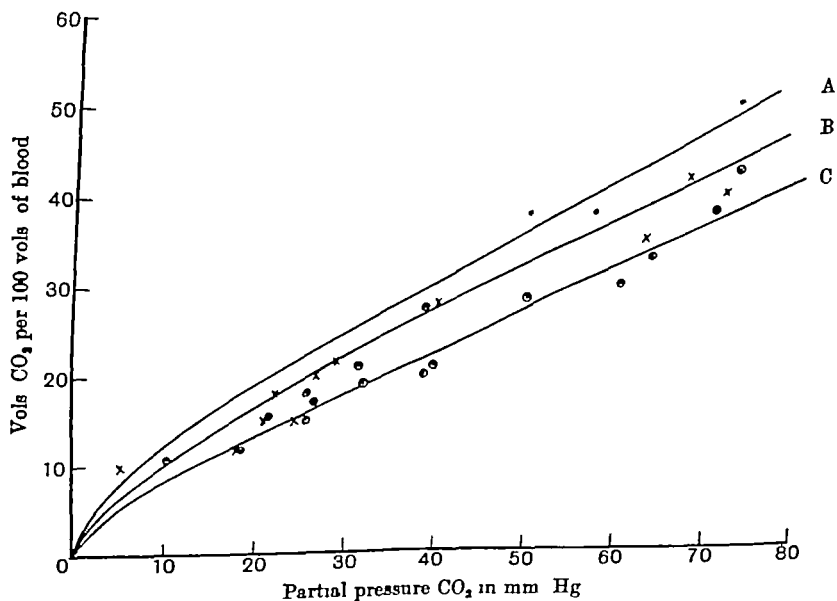


Fig 2 Fœtal carbon dioxide dissociation curve  
*OA* at 12.4 mm oxygen     $\times \times \times$  *OB* at 47.2 mm oxygen  
 ○ ○ ○ *OC* at 108 mm oxygen

points. In this way the average gas tensions in the foetus of the anæsthetised goat could be obtained. Three dissociation curves were made at average carbon dioxide tensions of 17.9 mm., 35.17 mm. and 66.5 mm. These curves are shown in Fig. 1.

Similarly three carbon dioxide curves were drawn at average oxygen tensions of 12.4 mm., 47.2 mm. and 108.4 mm. of oxygen. These appear in Fig. 2.

The average gas contents in Table I when applied to these curves yielded the gas tensions set out in Table III, which are the tensions of the gases in the arterial, venous and carotid bloods of the foetus.

TABLE III. The blood gas tensions of the foetus

	F.A.B	F.M.B	F.V.B
Oxygen in mm. Hg	41	28	15
Carbon dioxide in mm. Hg	44	54	61

It was not possible to determine these results entirely on one foetus owing to the technical difficulty of making the necessary curves on the one animal, and being certain at the same time that the physical conditions of the blood in the body and when shed did not markedly change during the experiment. Further, it was impossible to obtain any reliable results with this highly active young blood on one animal alone. Recourse therefore had to be made to the average method as described above. It will be noticed that the carbon dioxide tensions are quite high while the oxygen tensions are low. This is borne out by the colours of the bloods which in the urethanised goat are always dark red, even the foetal arterial blood being purple in hue.

*Measurement of Maternal Gas Tensions.* The indirect methods of blood-gas tonometry already used for determining the foetal blood tension were likewise used for the maternal bloods. The same average method was used as it was impossible to determine maternal and foetal gas tensions in one experiment.

The average quantity of gases of the arterial blood of the mother (urethanised) was

Oxygen	17.85 p.c.
Carbon dioxide	48.25 p.c.

The average percentage oxygen saturation was 82.6 p.c.

Dissociation curves were constructed in the same manner as for the foetal blood and the above results when applied to these curves yielded 60 mm. of oxygen and 43 mm. of carbon dioxide in the maternal arterial blood.

These results show that the difference in oxygen pressure between the maternal arterial blood going to the placenta and the foetal venous blood returning to the placenta is  $60 - 15 = 45$  mm of oxygen. This gradient is sufficiently big to suggest that oxygen can diffuse across the placenta from the mother to the foetus. There is no reason apart from histological consideration why one should hypothesise a secretory mechanism such as Bohr suggested.

In the case of carbon dioxide the difference in tension between the gas in the umbilical artery and the gas in the uterine artery is  $61 - 43 = 18$  mm. This margin of 18 mm is not so big as in the case of oxygen, but the diffusion constant for carbon dioxide across a wet membrane being much greater than that of oxygen, the gradient is more than enough for diffusion to carry out all the exchange of carbonic acid which is occurring.

### B Differential Tonometry

To investigate the question further recourse was had to a mode of experiment which might be called "Differential Tonometry." If two bloods *A* and *B* of equal volume and different dissociation curves are equilibrated against pure nitrogen in saturators of equal size, the resultant gas mixtures will have gas tensions proportional to the gas tensions in the original bloods. The gas tensions in the bloods will fall along the two curves *AA'*, *BB'*, while the gas tensions in the gas mixtures will rise along the curves *OA'*, *OB'* (Fig 3).

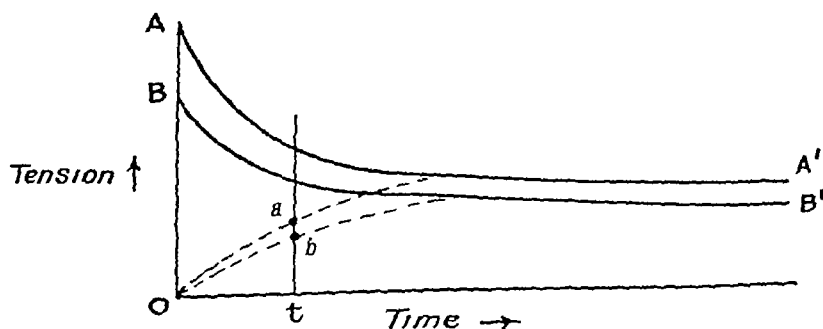


Fig 3

Therefore by measuring the gas tensions in the mixtures (*ab*) at any time *t* after equilibration was begun we obtain an index of the relative gas tensions at *A* and *B* initially. The advantage of measuring the percentage in the gas mixture is twofold. Firstly, equilibration can be

stopped at any time in the blood-gas mixture by simultaneously separating off the bloods from the gases, further variations in the blood gas tensions in one blood during analysis of the other do not cause any error, the gases remaining constant in composition once they are separated off. Secondly, it is easier to measure a slight difference in percentage in two gas mixtures with a Haldane gas analysis apparatus than it is to measure a similar difference in gas percentages in two bloods with the Van Slyke pump or any other blood-gas apparatus such as Barcroft's or Haldane's.

The objection to comparing the bloods directly by putting them in the separate bottles of a Barcroft differential manometer is that the Barcroft apparatus only gives differences in percentage saturation. If the bloods have the same dissociation curve this is the same as differences in gas tension. But if the bloods have different dissociation curves, as is the case with the maternal and foetal bloods, it might be quite possible for the more saturated blood to have a lower tension than the less saturated blood and so a result the exact reverse of the truth be obtained.

In carrying out this differential tonometry 10 c.c. each of the bloods to be compared were equilibrated against pure nitrogen in equal sized saturators for 10 minutes at 38° C. The bloods were separated off and the gases were analysed. The only fallacy liable to occur is one due to the Bohr effect, that is, supposing the carbon dioxide escapes from the blood into the nitrogen, the affinity of the blood for oxygen increases so that the oxygen tension does not fall in the blood nor rise in the gas mixture as much as it might if no carbon dioxide had escaped. The converse is equally true. This source of error is, however, unlikely to occur unless the gas tensions of the bloods to be compared are very close. This does not happen in these experiments.

In order to test this method a series of bloods containing gases at known tensions were made up and compared by measuring the percentages  $a$  and  $b$  (see Fig. 3) in the gas mixtures as described above. The results are given in Table IV.

In these experiments the time of equilibration varies, but the result is always the same, namely, that the gas tension is greater in the gas mixture which is in contact with the blood with the higher gas tension. Further, the method holds good for both carbon dioxide and oxygen. It must be borne in mind that it is not a method of measuring either the absolute tension of a gas in blood, nor is it a method of measuring the absolute ratio of the tensions in two bloods. As described

TABLE IV

Exp	Blood	Partial pressure		Relative pressures <i>a</i> and <i>b</i>	
		O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>
I	A	30	—	0 155	—
	B	60	—	0 313	—
II	A	10	20	1 19	1 54
	B	30	40	1 65	1 65
III	A	10	30	1 17	0 94
	B	24	48	1 69	1 03
IV	A	20	40	0 75	0 39
	B	40	20	1 47	0 29
V	A	20	40	0 428	0 664
	B	40	20	0 694	0 452
VI	A	30	—	1 075	—
	B	40	—	1 338	—
VII	Arterial blood from cat			2 69	0 766
	Venous blood from cat			1 61	0 807

above it merely indicates in which blood a gas (whether oxygen or carbon dioxide or both) has the higher partial pressure

In its present form it is not possible to use it for measuring absolute partial pressures, because to obtain these results consideration has to be given to the rates of diffusion of gases both in blood and in nitrogen, their relative solubility in different bloods, and the whole needs to be mathematically treated to give absolute results. This lies outside the immediate field of this research.

Applying this method of differential tonometry to the simultaneous tensions in maternal and foetal blood we obtain the results shown in Table V

TABLE V Relative blood gas tensions

I.	M.A.B oxygen	0 696	1 9
	F.V.B oxygen	= 0 366	= 1
	M.A.B carbon dioxide	0 76	1
	F.V.B carbon dioxide	= 0 92	= 1 21
II	M.V.B oxygen	1	
	F.A.B oxygen	= 1 3	

M.A.B = Maternal arterial blood.

F.V.B = Foetal venous blood, etc.

The first experiment shows that the gradients existing between the foetal venous blood going to the placenta and the maternal arterial blood going to the uterus are adequate for diffusion. The second experiment shows that the maternal venous blood in the uterine vein has a lower oxygen tension than the foetal arterial blood. This is not surprising if we remember that the placenta, unlike the lung, consumes an appreciable

quantity of oxygen and further that this venous blood comes not only from the placenta but also from the uterus

### C Reversal of Gases through Placenta

Zuntz in 1877 claimed to have reversed the passage of oxygen across the placenta so that the foetal venous blood had more oxygen than the foetal arterial blood as evidenced by the colour. That is to say, the mother on asphyxiation was withdrawing oxygen she had already given to the foetus. This could only occur in the case of a very acute and sudden maternal asphyxia such that the maternal arterial oxygen tension fell suddenly from above the foetal venous oxygen tensions to below it. It was found impossible to produce sufficiently acute an asphyxia to repeat this experiment, but by asphyxiating the mother it was possible to raise the carbon dioxide tension in the maternal blood so that the foetal venous blood absorbed carbon dioxide from the mother, as shown in Table VI, Exp IV

TABLE VI. Relative blood gas tensions.

III.	A. Normal		B After maternal rebreathing until foetal heart distressed	
	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
M.A.B	31	90	55	40
F.V.B	50	61	60	31

IV After maternal asphyxia until 3rd stage—foetus moribund			
	O <sub>2</sub>	CO <sub>2</sub>	
F.A.B	203	1183	
F.V.B	000	1062	

These experiments show that maternal rebreathing lessens the gradients, increases the absolute carbon dioxide tension in the mother and in the foetus, and decreases the absolute oxygen tension and in extremity causes a reversal of the carbon dioxide current across the placenta and marked inhibition of oxygen passage into foetus

It seems difficult to imagine that these effects could occur if the gases were secreted, whereas they are very easy to understand on a diffusion hypothesis

### SUMMARY

- (1) The foetal blood-gas percentage and tensions are measured in the urethamised goat
- (2) A method of differential blood-gas tonometry is described
- (3) The existence of a gradient from mother to foetus for oxygen and *vice versa* for foetus is demonstrated.

(4) It is shown that the gradient can be varied and with it the resultant foetal tensions

(5) The carbon dioxide current across the placenta is reversed

(6) The conclusion is reached that the gases diffuse across the placenta and are not secreted by placental activity

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# STUDIES ON THE INTERNAL SECRETIONS OF THE OVARY IV The significance of the occurrence of œstrin in the placenta

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## I INTRODUCTION

THE discovery that extracts capable of producing effects in ovariectomised animals could be prepared from ovaries soon led to the extraction of other organs, notably the placenta, with a view to ascertaining whether these also would yield extracts similarly active. The early workers on ovarian extracts were severely handicapped by the lack of accurate methods of testing, but nevertheless it was quickly found that effects brought about by the injection of ovarian extracts could also be produced by placental extracts. Fellner(8), for instance, showed that injection of alcohol-ether extracts of both ovaries and placentæ caused hyperplasia of the uterus in rabbits, and the same was noted of alcohol extracts of both organs by Herrmann(10) and of alcohol-acetone-ether-chloroform extracts by Seitz, Wintz and Fingerhut(17). Aschner(4) first showed that definite œstrous symptoms could be produced in ovariectomised animals by placental extracts.

The work of Allen and Doisy(1), who first dealt conclusively with the œstrus producing hormone of the ovary, and who first introduced the quantitative methods at present in use, led various authors to make similar extracts of placentæ with a view to obtaining the same hormone. Allen and Doisy themselves obtained large yields of œstrus producing substance<sup>1</sup> from placentæ, particularly from human. Dodds, Dickens and Wright(6) obtained definite though lesser yields. Similar results

<sup>1</sup> In view of their identical biological action there seems little or no reason to doubt that the œstrus producing substance obtainable from placentæ is identical with that obtained from ovaries, and in this paper the identity will be assumed.



were recorded by Frank and Gustavson<sup>(9)</sup>, and these authors, assuming that the œstrus producing hormone represented the entire mechanism of ovarian secretion, were led to elaborate their theory of the "gestational gland"

It is quite evident, therefore, that œstrin (which we have elsewhere<sup>(15)</sup> suggested as a suitable term for the œstrus producing hormone) can be obtained from the placenta, and this fact presents a very curious problem. Allen, Pratt and Doisy, in a recent paper<sup>(2)</sup> discuss the significance of the presence of œstrin in the placenta, and tend to suppose that it is elaborated there. Combining this hypothesis with their supposition that œstrin is elaborated by the human corpus luteum, they tend to agree with Frank and Gustavson<sup>(9)</sup> who maintain that the œstrus producing hormone of the ovary is the dominating factor in all phases of the œstrous cycle, including pregnancy, and is produced firstly by the follicle, then, after the rupture of the follicle, by the corpus luteum, and finally by the placenta. Our own results do not, however, support this view. In the first place the elaboration of œstrin in the ovary is clearly not a prerogative of the Graafian follicles (5, 14, 15), and so the capacity of the ovary to elaborate it is not necessarily dependent on the presence of mature follicles. Hence, the elaboration could be carried on at all stages of the cycle without the interference of the corpora lutea and placenta. Secondly, turning to the corpus luteum, in our experience of cow corpora œstrin is only present in appreciable quantities in the fluid which is found inside hollow specimens, and which is probably homologous with *liquor folliculi*. It is hoped shortly to publish these observations, but in the meanwhile it may be pointed out that this finding is all against the supposition that œstrin is elaborated by the corpus luteum. The implication is that such œstrin as may be found in corpora lutea is there accidentally. In addition the work of Loeb<sup>(12)</sup>, Papanicolaou<sup>(13)</sup> and our own observations<sup>(16)</sup> have conclusively demonstrated an œstrus inhibiting action on the part of the corpora lutea under certain conditions, and it is hardly credible that they should simultaneously combine the two functions of œstrus promotion and inhibition. The fact that under normal circumstances œstrus never occurs during pregnancy suggests immediately that œstrus is incompatible with normal gestation, and we have in fact shown elsewhere that the artificial production of œstrus during pregnancy by the injection of œstrin leads to re-absorption or abortion. It seems, therefore, highly improbable that the œstrin found in the placenta is elaborated there for the placenta to elaborate and secrete œstrin would, in fact, be a definite attempt at suicide. In short,

while not wishing to deprecate for one moment the great value of the work, particularly that of Allen, which has been done on the œstrous hormone, we would register a protest against the present tendency to regard it as the factor concerned in the development of the secondary organs and as the sole regulator of the female reproductive cycle of diœstrus, œstrus, pregnancy and lactation

If, however, it is assumed as a tentative working basis that œstrin is not elaborated by the placenta, it is necessary to account for its occurrence there in far greater amounts than it is found in any other organ (excluding the ovary) in the body<sup>1</sup> In this connection Lillie's (11) remark that the foetuses must be protected in some way from the sex-hormones of the mother seemed to have a possible significance

It is well known that the ovarian stimulus necessary for the original development of the reproductive organs is also necessary during adult life for the maintenance of those organs, and is therefore present during adult life It is also known that this stimulus is *hormonic* in nature Further, Lillie has shown (11) that the subjection of a female foetus to the *hormonic* influence of a male foetus results in intersexual development, leading, in the case of the cow, to the animal known as the "freemartin." Considering these facts it is not unreasonable to suppose that if the male foetuses in the normal pregnant animal were not by some means protected from the sex hormones of the mother aberrations of sexual differentiation would occur It is reasonable to infer, therefore, that some mechanism exists whereby the sex hormones of the mother are prevented from reaching the foetuses In our view it is improbable that œstrin is the actual hormone associated with the original development of the accessory sexual organs of the female, but at the same time the above argument suggested very strongly that the concentration of œstrin in the placenta, where we are assuming it is not elaborated, might be the result of some protective retention on the part of the placenta In other words, it seemed possible that the placenta might withdraw œstrin from the circulation in order that the foetuses might thereby be protected This view becomes more probable when it is considered that the statement that œstrin can be extracted in comparable amounts from both ovaries and placenta is quite misleading as regards the gross amounts obtainable from the two The mouse units per kilogram obtainable from both may be put at about the same average figure, but the weight of the

<sup>1</sup> While it has not been shown that *no* œstrin can be extracted from other organs, it is fairly certain that amounts comparable to those found in the ovary and placenta cannot be obtained from other sources

pair of ovaries of the cow is about 10 grm while the weight of the full time placental cotyledons of the cow is about 5000 grm This means that the actual number of mouse units contained in the placenta is 500 times the number obtainable from the two ovaries Hence, if the amount which can be obtained from the ovaries represents only 12 hours' secretion, the entire ovarian output could be retained by the placenta for 8 months, or nearly the entire gestation period of the cow

If, however, the placenta merely withdraws œstrin from the maternal circulation one would expect, as originally suggested by Allen, Pratt and Doisy, that the hormone would not actually cross the placenta, and that it would be concentrated in the maternal rather than in the foetal tissue of the organ Further, it was clear that this hypothesis could be tested in cases where the maternal and foetal tissues could be easily separated for separate extraction Discoid placentæ, such as are found in the human subject, offer but little chance of effecting such a separation, but in the cotyledonous placentæ of ruminants such as the cow and sheep, separation and, as originally suggested by Allen, Pratt and Doisy (2), extraction of the maternal and foetal portions of the cotyledons presents a comparatively simple task In actual practice the separation was found to be easier in the cow owing to the larger size and to the fact that in this animal the maternal tissues are much paler in colour than the foetal Gentle pulling easily brings the two sides apart Such a separation cannot, of course, claim to be absolutely complete, but it is undoubtedly sufficiently so for the purpose in view The main part of the present paper consists, therefore, of comparisons of the yields of œstrin from the maternal and foetal sides of cow placental cotyledons In addition some further material is presented relating to the yields of œstrin obtainable from the human placenta, and to the testing of uterus tissue, amniotic fluid and whole fetuses for the presence of the hormone

## II METHODS AND MATERIAL

The extracts were prepared in essentially the same manner as those described in earlier papers, modifications being introduced only as required by the nature of the tissue The human placentæ were put into alcohol in the wards as soon as available in batches of one, two or three Owing to this method of collecting, the weights obtained were for the partially dehydrated and coagulated tissue The cow and sheep placentæ were received frozen, and after being allowed to thaw out the cotyledons were cut from the uterus and membranes, separation of the foetal and

maternal sides being first performed in the case of the cow. The foetuses were minced whole, and the uterine tissue was freed as carefully as possible from placenta. In the case of the amniotic fluid, of which as much as 6000 c.c. went to one extract, it was thought desirable to concentrate the bulk down before adding the alcohol. By this means the volume of fluid for refluxing was kept within reasonable bounds.

In all cases the extraction was as previously outlined, the ordinary alcohol-ether-acetone-alcohol sequence being used. Where the yield was considerable only a known proportion was emulsified for testing, in most of the cases of placenta the amount was 1/10 of the whole. In all cases the amount to be given was injected at one time. The technique of testing has been previously discussed and need not be referred to again. A mouse unit is, of course, the least amount of an extract necessary to produce oestrus.

Since both the amount that will emulsify in a given amount of carbonate and also the amount of emulsion that can be injected at one time is strictly limited the amount of an extract which can be injected as emulsion is also limited, varying in our experience from 50–100 mg. This means that a given extract can rarely be tested down to one mouse unit. This, however, is not a great disadvantage. For instance, 500 gm. of tissue, giving a yield of 1.5 gm., could not be tested for less than 30 M.U.K. without purification. Such low yields as these, however, are rarely found from ovaries and placenta, and in other tissues it is possible by simple purification of the crude extract to test down to about 10 M.U.K. Since this activity is almost negligible, extracts inactive to a detectable degree are said to contain less than a given number of M.U.K. (i.e. less than the least number it was found possible to test for). Owing to their lesser content of solid matter, fluids (liquor folliculi, amniotic fluid, etc.) are more readily tested for low values.

The following abbreviations are used through this paper

G.K.	Yield in grams per kilogram of tissue	} Calculated to the nearest unit
M.U.	Number of mouse units	
M.U.K.	Mouse units per kilogram	
M.U.G.	Mouse units per gram yield	

### III EXTRACTION OF WHOLE PLACENTA

*Sheep* The cotyledons of the uteri from 7 pregnant sheep were extracted in 4 batches, one batch containing the cotyledons from four uteri. The following results were obtained

TABLE I. Yields from sheep placenta

No of extract	Weight of tissue gram	Crude yield gram	G K.	M U	M U K	M U G
P 1	930 (1)	2 05	2 20	170	183	83
P 3	580 (1)	1 68	2 88	115	198	68
P 4	3110 (4)	7 82	2 52	360	110	46
P 5	650 (1)	1 25	1 92	200	308	160
	5270 (7)	12 80	2 43	845	161	66

The foetuses from these uteri varied from 200–300 mm in length, so that they were probably in the 3rd and 4th months of gestation

*Cow* Ten extractions of cow cotyledons are available. In each case the foetal and maternal sides were extracted separately, but calculated as whole placentæ the results were as follows

TABLE II. Yields from cow calculated as whole placentæ

No of extract	Weight of tissue gram.	Crude yield gram	G.K.	M U	M U K.	M U G
P 2	1800	6 47	3 59	345	192	54
P 6	4150	17 40	4 19	1373	331	79
P 7	1590	5 65	3 56	1130	711	200
P 16	3280	16 02	4 88	1550	473	97
P 28	1960	10 09	5 46	710	362	66
P 30	360	1 49	4 14	765	2123	514
P 31	2660	12 34	4 64	760	286	62
P 32	1500	6 59	4 39	440	293	67
P 37	2360	8 49	3 60	515	218	60
P 38	1970	9 78	4 96	510	259	52
Total	21630	94 92	4 39	8098	374	85
Average per uterus	2163	9 492		809 8		

*Human placenta* Thirty-six human placentæ have been extracted in 20 batches, 7 batches containing only 1, 10 batches 2 and 3 batches 3 placentæ. The results are given in Table III

For human placenta, therefore, the average weight is 424 gram, the average yield 98 gram, and the average number of mouse units 122

*Comparison of yields from maternal and foetal sides of cotyledons* The data for the yields from the maternal and foetal sides of the cow cotyledons are given in Table IV

#### IV EXTRACTION OF UTERUS, FŒTUS AND AMNIOTIC FLUID

*Amniotic fluid* Three extractions of amniotic fluid (1 cow, 2 sheep) were made, the original material being evaporated down under reduced pressure to about one-quarter of its original volume before addition of alcohol. As would be expected, the yields of material were very small,

TABLE III Yields from human placenta

No of extract	Weight of tissue gram	Crude yield gram	G. K.	M. U.	M. U. K.	M. U. G.
P 8	460 (1)	1.75	3.81	200	435	114
P 9	440 (1)	1.23	2.79	250	568	204
P 10	940 (2)	1.52	1.62	62	66	41
P 11	1020 (3)	1.56	1.53	166	163	106
P 12	320 (1)	.88	2.75	230	719	261
P 13	980 (2)	1.13	1.15	69	71	62
P 14	900 (2)	3.01	3.35	176	195	58
P 15	920 (2)	1.60	1.74	270	293	175
P 17	400 (1)	1.05	2.62	125	313	119
P 18	420 (1)	1.37	3.26	50	120	36
P 19	760 (2)	2.11	2.78	255	336	121
P 21	930 (2)	1.73	1.86	83	89	48
P 22	520 (1)	1.95	3.75	82	158	42
P 24	820 (2)	1.35	1.64	160	195	118
P 25	1150 (3)	1.90	1.65	480	418	252
P 26	440 (1)	1.91	4.34	140	318	73
P 27	800 (2)	2.97	3.71	582	728	196
P 34	980 (2)	2.44	2.49	277	283	115
P 35	1080 (3)	2.22	2.05	500	463	225
P 36	980 (2)	1.78	1.82	245	252	138
Total	15260 (36)	35.46	2.32	4402	288	124
Average per placenta	424	.98		122		

TABLE IV Comparison of yields from maternal and foetal sides of cotyledons

No of extract	Wt of tissue		Crude yield		G. K.		M. U.		M. U. K.		M. U. G.	
	Ma gram	Foetal gram	Ma gram	Foetal gram	Ma ternal	Foetal	Ma ternal	Foetal	Ma ternal	Foetal	Ma ternal	Foetal
P 2	750	1050	2.93	3.54	3.91	3.37	180	165	240	157	61	47
P 6	2400	1750	10.30	7.10	4.29	4.05	486	887	203	506	47	126
P 7	840	950	2.85	2.80	4.45	2.94	570	560	891	597	200	201
P 16	1820	1460	7.47	8.55	4.12	5.88	1040	510	572	350	139	59
P 28	1120	840	6.37	4.32	5.69	5.14	590	120	527	143	93	28
P 30	200	160	.96	.53	4.80	3.31	640	125	3200	782	667	236
P 31	1620	1040	7.40	4.94	4.57	4.75	500	260	308	250	68	53
P 32	480	1020	3.02	3.57	6.29	3.50	310	130	646	127	102	36
P 37	1220	1140	5.00	3.49	4.09	3.06	390	125	319	110	78	35
P 38	1010	960	6.53	3.25	6.47	3.38	280	250	257	261	40	77
Total	11260	10370	52.83	42.09	4.69	4.06	4966	3132	441	302	94	74

TABLE V Yields from amniotic fluid.

No of extract	Volume of fluid c c	Yield gram.	G. K.	No activity detected			Activity detected		
				In active m gram	Tested down to		M. U.	M. U. K.	M. U. G.
					M. U.	M. U. K.			
A F 1 (sheep)	550	20	363	.08	<2.5	<4.5	—	—	—
A F 2 (cow)	6000	85	142	—	—	—	90	15	106
A F 3 (sheep)	500	.09	180	—	—	—	25	50	278
Total of 2 and 3	6500	.94	146	—	—	—	115	18	125

but in two cases it was possible to detect œstrus producing activity. The actual results are given in Table V.

It is curious that the first of these batches of amniotic fluid, though giving the highest crude yield, should have no detectable activity (any activity it possessed being  $< 4.5 \text{ M U K}$ ) while the last two should have comparatively good yields. In the last two the  $\text{M U G}$  is high, but the  $\text{M U K}$  is low in correlation with the low yields of extract. The total amniotic fluid extracted has the following values: Volume of fluid 7050 cc, yield, 1.14 cc,  $\text{G K}$ , 164,  $\text{M U}$ , 115–117.5,  $\text{M U K}$ , 16–17,  $\text{M U G}$ , 101–103.

*Fœtus* Two extractions of whole sheep fœtus have been made. No œstrin was detected in either of these. The details are as follows.

TABLE VI. Extracts of sheep fœtus

No of extraction	Amount of tissue gram	Yield gram	$\text{G K}$	Inactive in gram	Tested down to		
					$\text{M U}$	$\text{M U K}$	$\text{M U G}$
F 1	310	1.02	3.29	20	$< 5$	$< 16$	$< 5$
F 2	280	.83	2.96	21	$< 4$	$< 14$	$< 4$
Total	590	1.85	3.13	—	$< 9$	$< 15$	$< 5$

These results show that œstrin, if present at all in sheep fœtuses, is present in very small amounts only.

*Uterus* Pregnant uteri from 1 cow and 2 sheep have been extracted. The results are given in Table VII.

TABLE VII. Extraction of uteri.

No of extract	Amount of tissue gram.	Yield gram		G K. (crude)	No activity detected			Activity detected		
		Crude	Purified		In active in gram	Tested down to		M U >1	M U K.	M U G (crude)
						M U	M U K.			
U 1 (sheep)	1500	4.80	.82	3.07	—	—	—	<20	<13	<4.3
U 2 (cow)	500	.93	—	1.86	.09	<10	<20	—	—	—
U 3 (cow)	1650	3.10	.43	1.94	—	—	—	75	45	24

In the case of U 1,  $1/20$  of the material was first injected. This proved inactive, and the remainder was purified by freezing in methyl alcohol. The purified product was remarkably fluid and was injected direct without emulsification. The animal died 4 days later, but showed œstrous symptoms first. Of the other two extracts one showed quite a pronounced activity. How far this occurrence of œstrin in the uterine tissue, and how far the observed variation in activity, are due to the incomplete removal of the œstrin-containing cotyledons is uncertain, but it is difficult to suppose that the comparatively high activity of U 3 was solely due to retained cotyledon tissue.

V DISCUSSION

*Comparison of yields with those recorded by other authors* The tables given above show that the 42.16 kg of placenta extracted gave an average yield of 317 M U K, and that the 143.18 gram of "crude hormone" had an average activity of 93 M U G. Compared with this average the human placental extracts give a lower M U K. (288) but are purer (M U G 124). The M U K yield is low compared with those reported by Allen and Doisy and their collaborators. The number of rat units per kilogram reported by these authors for human placenta are as follows: 700, 400 and 580 (7), 236 and 302 (2). The exact relation between a rat unit and a mouse unit is uncertain, but according to Dr Doisy<sup>1</sup> it is at least 4:1. In this case their yields are very considerably above our own. The one extract of placenta made by Dodds, Dickens and Wright (6), however, gave only 85 rat units per kg.

*Comparison of placental with ovarian yields* The yields from placenta compare with those previously obtained by us from ovaries as follows:

TABLE VIII. Comparison of placental and ovarian yields

Tissue	Amount of tissue	Crude yield				
		gram.	G.K.	M U	M U K.	M U G
Ovarian stroma	4780 gram.	16.88	3.50	1019	213	60
Liquor folliculi	3383 (c c)	3.05	90	422	125	138
Whole ovary	3120 gram.	6.92	2.22	830	266	120
Placenta	42160 gram.	143.18	3.39	13345	317	93

Comparing these results it is seen that the G.K. is, as might be expected, lowest with liquor folliculi (though far from as low as the amniotic fluid value) while ovarian stroma and placenta give the highest G.K., whole ovary occupying an intermediate position. On these figures the placenta gives a rather higher yield than ovary itself. The purity of the extract is naturally greatest with liquor folliculi, and low with ovarian stroma and placenta.

*Specific variation in yields from placenta* The following table shows the variation in the yields from the placenta of different species:

TABLE IX. Specific variation in yield.

Animal	Weight of tissue		Crude yield			
	gram.	gram.	G.K.	M U	M U K.	M U G
Sheep	5270 (7)	12.80	2.43	845	161	66
Cow	21630 (10)	94.92	4.39	8098	374	85
Human	15260 (36)	35.46	2.32	4402	283	124
Total	42160 (53)	143.18	3.39	13345	317	93

<sup>1</sup> I am much indebted to Dr E. A. Doisy for a private communication on this point.



This table shows that the yield of "crude hormone" per kilogram of tissue is very much the same in the case of the sheep and the human, while cow placenta give a crude yield of some 80 p c more. This crude yield is, however, nearly twice as active in the case of the human placenta as in the case of sheep. The highest M U K is given by the cow.

What these specific differences mean, if anything, is difficult to say. The human material differs, of course, from the other in being all full term placenta, but as no correlation of yield with age of placenta (i.e. size) can be seen in the cow, this point is apparently unimportant.

*Variation of yields from different batches of same species of placenta*  
In Table X is shown the different yields from different batches.

TABLE X

Nature of tissue and number of batches	Range of G K yields	Range of M U K yields	Range of M U G yields
Sheep (4)	1.92-2.88	116-308	68-160
Cow (M) (10)	3.91-6.47	203-3200	40-807
Cow (F) (10)	2.94-5.86	110-782	28-236
Human (20)	1.15-4.34	66-728	36-261

The variation in yield of the different batches is very great, especially as regards the M U K. The actual range of variation in the batches of one species as compared with those of another species has little significance on account of the difference in the number of batches. The material is, of course, not sufficiently extensive to allow of statistical treatment.

*Comparison of yields from maternal and foetal sides of cow placenta*  
Table XI gives the actual yields from the maternal and foetal parts of cow placenta. The following table gives the value of maternal/foetal yield in each case.

TABLE XI Comparison of yields from maternal and foetal sides of cow placenta

No	Wt of tissue M/F	Crude yield M/F	G K M/F	M U M/F	M U K M/F	M U G M/F
P 42	71	83	1.16	1.09	1.53	1.30
P 6	1.37	1.45	1.06	.55	.40	1.37
P 7	.67	1.02	1.51	1.02	1.49	.90
P 16	1.24	.87	.70	2.04	1.63	2.36
P 28	1.33	1.47	1.11	4.92	3.68	3.32
P 30	1.25	1.81	1.45	5.12	4.09	2.83
P 31	1.56	1.50	.96	1.92	1.23	1.28
P 32	.47	.85	1.80	2.48	5.09	2.83
P 37	1.07	1.43	1.34	3.12	2.90	2.23
P 38	1.05	2.01	1.91	1.04	.98	.52
Total	1.08	1.26	1.16	1.59	1.46	1.27

From this table the following observations may be made

- (a) In 7 out of the 10 cases the maternal tissue exceeds the corresponding foetal in gross amount
- (b) In the same 7 also, the gross yield from maternal is greater than from foetal tissue
- (c) In all cases, except one, the gross yield in M T is greater from the maternal side
- (d) In 8 out of the 10 cases the M T K is greater from the maternal side
- (e) The maternal extracts are more active in 7 of the cases

*The distribution of œstrin in reproductive tissues* It remains now to consider what light is thrown by these experiments on the distribution of œstrin in the organs concerned in reproduction and in turn what light this distribution throws on the source of origin

In the first place since the above table shows that placental extracts have much the same order of activity as ovaries themselves the failure to obtain comparable yields from other products of conception and from uterine tissue is noteworthy. The actual values are compared below

TABLE XII. Comparison of yields from placenta, amniotic fluid, foetus and uterus

Nature of tissue	Amount of tissue gm.	Crude yield gm.	G.E.	M T	M.T.K	M.T.G
Placenta	42160	143.18	3.39	13345	317	93
Uterus	3650	8.63	2.40	<105	<29	<12
				>76	>21	>9
Amniotic fluid	7050	1.14	16	<117	<17	<101
				>115	>16	>103
Foetus	590	1.83	3.13	<9	<15	<5

In addition Allen, Pratt, and Doisy<sup>(2)</sup> have reported that active extracts can be prepared from curetted material, chorionic vesicle, umbilical cord and amniotic fluid. Human embryos of the 2nd, 3rd and 4th months<sup>(3)</sup> and a pig embryo<sup>(4)</sup> all gave negative results. The strange position is found, therefore, that œstrin has been extracted from ovaries, uterus and every product of conception except the embryo and foetus. This conclusion does not definitely support the hypothesis that the placenta has a protective ab-orptive function, and not one of elaboration. At the same time however it is scarcely possible to assume that it is elaborated in the amniotic fluid, and its presence there is further evidence that it is not necessarily elaborated by the placenta because it is found in the placenta. In this connection it may be urged that since the œstrin content of the ether-acetone soluble constituents of the amniotic fluid (M.T.G = 125) is nearly as high as that of liquor folliculi (M.T.G = 138),

the liquor amni may have absorbed as much as it can hold in colloid solution. Its occurrence in other products of conception than the placenta may, in fact, indicate a necessity for extending the absorptive capacity of the placenta. To sum up, it may be said that while the results recorded above offer no conclusive proof that the placenta merely absorbs oestrin from the maternal system, they do increase the improbability that it is actually elaborated by the placenta.

## VI SUMMARY

(1) It is suggested that the presence of oestrin in the placenta is more easily explained on the grounds that the placenta withdraws oestrin from the maternal circulation to protect the foetuses from its action, rather than on the grounds that the placenta elaborates the hormone.

(2) With a view to throwing light on this hypothesis the maternal and foetal sides of cow placental cotyledons were extracted separately, but oestrin was found to be fairly equally divided between the two.

(3) In addition active extracts have been prepared from uterine tissue and amniotic fluid. In each case two out of three extracts gave positive results. The remaining extract of each, however, gave negative results.

(4) It is concluded that while these results afford no conclusive proof that oestrin is merely absorbed from the maternal circulation by the placenta, they do lessen the probability that it is elaborated by the placenta.

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# THE NATURE OF THE VASO-DILATOR CONSTITUENTS OF CERTAIN TISSUE EXTRACTS

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It has long been known that simple watery or alcoholic extracts of various organs of the body produce a pronounced depressor action when injected intravenously, especially in the carnivora. Oliver and Schafer<sup>(1)</sup> observed such an action with an extract of thyroid gland, Mott and Halliburton<sup>(2)</sup> with extracts of brain and nervous tissues, Vincent and Sheen<sup>(3)</sup> with extracts from liver, muscle and various glandular organs, and other observers with a wide range of organs from different species. The suggestion that these effects were explained by the presence of choline in such extracts was shown to be inadequate by Vincent, with Osborne<sup>(4)</sup> and Sheen<sup>(3)</sup>, who found that the action was but little affected by administration of atropine. Popielski<sup>(5)</sup> suggested that a common depressor agent, for which he suggested the name "vaso-dilatin," was present in such tissue extracts, and a hint appeared to be given as to its chemical nature when Dale and Laidlaw<sup>(6)</sup> found that the base now generally known as "histamine" produced the vascular and other characteristic effects of the hypothetical vaso-dilatin, when injected in relatively minute doses. Barger and Dale<sup>(7)</sup> soon afterwards succeeded in isolating from an extract of intestinal mucous membrane sufficient of this base for chemical as well as physiological identification. The quantity obtained, however, was small in relation to the total activity of this kind exhibited by the extract, and the nature of the raw material left it doubtful whether the histamine came from the living cells of the mucous membrane, or from the intestinal contents with which they had been in contact. For some years after this, though the correspondence in action, between the chief depressor constituent of various organ extracts and histamine, was widely recognised, the probability of their chemical identity seemed rather to be weakened. We may mention, for example, the observations of Stern and Rothlin<sup>(8)</sup>, who extracted from the spleen a principle acting like histamine, but, finding that it was largely destroyed by heating with dilute alkali, concluded that it could

not be histamine, which is stable to such treatment. We shall show later the need for caution in drawing such conclusions, but the balance of evidence and of opinion seemed to be unfavourable to the identification until Abel and Kubota published an important paper in 1919<sup>(9)</sup>, in which they claimed to have made probable the presence of histamine itself, or of some substance closely related to it, as the essential depressor constituent of extracts from a number of organs and tissues, and of the products of partial hydrolysis of pure proteins. In some respects their evidence failed to be completely convincing. From two tissues only—the pituitary gland and the gastro-intestinal mucous membrane—did they obtain pure salts, the picrate and chloroaurate, of histamine. The quantities so isolated were small in relation to the original depressor activity, the pituitary raw material was a commercial dried preparation of the whole gland, and the considerations above mentioned left a doubt as to the significance to be attributed to the presence of a small amount of histamine in an extract from material which included intestinal mucous membrane. In all other cases they were unable to make the final purification needed for chemical recognition. They showed that a depressor substance was present which, when dried with sodium carbonate, could be extracted with hot chloroform, and which had actions like that of histamine on the blood-pressure and on plain muscle, but such evidence could do no more than make it probable that something having similar solubilities and similar activity to those of histamine was present, it would not justify a general conclusion that histamine itself was the main depressor constituent of the tissues.

Our own renewed investigation of the chemical nature of the depressor substance has been prompted partly by a growing conviction of its physiological significance, and partly by recent claims made for the therapeutic value of depressor constituents of the liver, in particular. Papers by one of us (Dale) with Richards<sup>(10)</sup>, Laidlaw<sup>(11)</sup> and Burn<sup>(12)</sup> have given grounds for, and indicated the trend of, the physiological speculations. Another of us (Best) had taken part in experiments on the production and testing of the liver extracts, used in one group of the recent observations on their therapeutic effects. Our attention was, for this reason, first directed to the identification of such vaso-dilator substances, in liver extracts, as had a clearly recognisable type of immediate action. Histamine itself, together with relatively large amounts of choline having been isolated from the liver extract, we extended the observations to another organ extract, that of lung, which exhibits a peculiarly intense histamine-like action.

The results obtained with these two tissues seem so significant that it appears desirable to publish them now, leaving for future publication the result of the investigation of other organs. It should be made quite clear at the outset, however, that our findings must not be assumed to be applicable to every case in which the intravenous injection of an extract of any organ has been found to lower the systemic arterial pressure. Evidence has long been available which forbids the attribution of some such effects to histamine or to choline, or to both acting together. As Vincent and Curtis<sup>(13)</sup> have recently pointed out, Vincent and his earlier co-workers obtained depressor effects by injecting extracts of brain matter into atropinised rabbits. Vincent and Curtis<sup>(13)</sup> state that they have again obtained such depressor effects with organ extracts in atropinised rabbits. Since atropine annuls the action of choline, while the rabbit under ether or urethane is not sensitive to the vaso-dilator action of histamine (Dale and Laidlaw), these effects were clearly of another type. We are not here concerned with them. Our purpose has been to examine the nature of the substance producing the histamine-like action, so widely prevalent in organ extracts, and, since we found it in association with the other, of that producing the weaker, choline-like action. The physiological experiments have been carried out by Best and Dale, while Dudley and Thorpe have been responsible for the chemical side of the investigation, but the two kinds of technique have been used in close co-operation at all stages.

#### PHYSIOLOGICAL ANALYSIS OF THE ACTION OF LIVER EXTRACTS

The experiments under this heading formed the real starting-point of the investigation. One of us (C. H. B.) had been engaged in Toronto with D. A. Scott in the preparation and testing of liver extracts for the therapeutic investigation above mentioned. A quantity of extract was prepared here by a method and under conditions practically identical with those which were being used in the Connaught Laboratories, Toronto. The opportunity of having this preparation carried out on a large scale we owed to the British Drug Houses, Ltd., and especially to the friendly and expert co-operation of their technical director, Mr F. H. Carr, and his staff. The details are given below in the chemical section. Here it need only be mentioned that the final procedure used in purifying the extract, viz. the precipitation of the depressor constituents from solution in 80 p.c. alcohol by adding 3 volumes of acetone, appeared to render it highly improbable that they were simple bases like histamine and choline. Neither of these substances, in concentrations of the order

indicated by the activity of the extracts, is precipitated at all from pure solution under these conditions

Nevertheless a few orientating experiments soon established the fact that the immediate, depressor action exhibited by such extracts, when injected intravenously into the anaesthetised cat or dog, was chiefly of the histamine type. In a cat anaesthetised with ether, by measuring the evanescent effects of small doses of the order of 0.01 c.c., it was easy to balance the effects against those of small doses of the order of 0.001 mg of pure histamine<sup>1</sup>. The equivalents having thus been determined in terms of depressor action, the solutions were then compared for their stimulant activity on the plain muscle of the isolated uterus of the guinea-pig. Their activities in this direction showed a practically identical relation.

*Exp 1* Cat anaesthetised with ether. Vagi cut. Arterial pressure from carotid. Intravenous injection. Liver extract (L.E. No. 1) diluted 10 times compared with a solution of histamine containing 0.01 mg of the base in 1 c.c. A number of trials gave a final equivalent

$$\begin{aligned} 0.1 \text{ c.c. diluted liver extract} &= 0.12 \text{ c.c. histamine solution} \\ &= 0.0012 \text{ mg histamine} \end{aligned}$$

1 c.c. L.E. No. 1 contains the depressor equivalent of 0.12 mg histamine.

*Exps 2 and 3* Isolated uteri of virgin guinea pigs. Equivalents were obtained indicating

$$\begin{aligned} 7 \text{ c.c. of extract No. 1} &\text{ are equivalent to } 1 \text{ mg histamine,} \\ 9 \text{ c.c. of extract No. 1} &\text{ are equivalent to } 1 \text{ mg histamine} \end{aligned}$$

Average 8 c.c. of extract No. 1 are equivalent to 1 mg. of histamine,  
or 1 c.c. of extract No. 1 is equivalent to 0.125 mg histamine

A more specific test of the histamine-like nature of the action was afforded by the uterus of the rat, which, as Guggenheim<sup>(14)</sup> showed, is peculiar in the fact that the tone and rhythm of its plain muscle are inhibited by histamine, which must be applied in relatively large doses to produce any definite effect. In this respect again the action of the liver extract corresponded with that of histamine. The quantitative relation could not be clearly determined, and there were indications that the inhibitor, histamine-like action was complicated by the presence in the extract of something having a weak stimulant action, but the main action was clearly of the histamine type.

We further tested the action of the extract by injecting it intravenously into unanaesthetised guinea-pigs. In young guinea-pigs weighing about 200 grm an injection of 0.05 mg of histamine usually,

<sup>1</sup> The preparation used for comparison was the crystalline diphosphate, sold as "Frigamine diphosphate" by Burroughs, Wellcome and Co. but the solutions were made up and doses expressed in terms of histamine base.

but not quite invariably, produces death from asphyxia, due to constriction of the bronchioles, 0.1 mg is an invariably fatal dose. The lethal dose may, therefore, be placed somewhere between these two limits. 0.02 mg never produces noteworthy symptoms.

*Exp. 4.* The extract used in this experiment (L.E. No. 2) had been assayed on a cat under ether, in comparison with histamine, small doses being used, with the result that the undiluted extract was calculated to be equivalent in depressor action to a histamine solution containing about 0.1 mg of the base per c.c. Five guinea pigs, weighing about 200 gm. each, were given intravenous injections, with results as follows:

Dose	Result
(1) 0.1 mg histamine	† 6 minutes
(2) 0.1 mg histamine	† 3½ minutes
(3) 0.8 c.c. L.E. No. 2	† 4 minutes
(4) 0.2 c.c. L.E. No. 2	No symptoms
(5) 0.75 c.c. L.E. No. 2	† 4 minutes

The symptoms produced by histamine and the effective doses of the extract were identical. The autopsies also showed the same picture in all the cases in which death resulted. The heart continued to beat for some minutes after complete cessation of respiratory and other movements. The lungs showed the characteristic permanent distension, and there was no other definite abnormality to suggest that any action was produced by the extract which was not identical with that of histamine. It will be seen, further, that the lethal effect on guinea pigs corresponds with what would be expected from its depressor equivalent. A dose of extract equivalent in depressor action to 0.02 mg of histamine produces no obvious symptoms, while doses equivalent to 0.075 and 0.08 mg produced asphyxial death with approximately the same rapidity as 0.1 mg of histamine itself.

This evidence suggested that a substance acting like histamine not only contributed to the depressor action of the extract, as measured by the immediate, evanescent effect on the cat's blood-pressure, but was the predominant agent in its production. No indication, indeed, had thus far been obtained of the presence of any differently acting depressor substance. A further test on this point, however, could be obtained by observing the action of the extract on the artificially perfused blood vessels of the cat's leg. These vessels readily lose their vaso-dilator response to histamine under these conditions, while retaining their responsiveness to vaso-dilators of other types. We accordingly carried out a series of artificial perfusions of cats' hind legs, with defibrinated cat's blood, or, in some cases, with Locke's solution containing gum and a small proportion (1 in 10 millions) of adrenaline. The apparatus used for perfusion, and that for recording venous outflow and limb volume, were the same as those described by Burn and Dale<sup>(12)</sup>. In the early stages of the perfusion we found, as they had done, that a small dose of histamine, injected into the arterial cannula, produced a definite vaso-dilatation. Even at this stage, however, a dose of the liver extract, which



had been found to produce an equal fall of arterial pressure in the anæsthetised cat, produced a definitely greater vaso-dilatation in the perfused organ, as shown both by a more pronounced acceleration of the venous outflow, and by a greater increase of the limb volume. This indication of the presence in the liver extracts of some other vaso-dilator principle became much clearer, when the stage of the experiment was reached at which histamine no longer produced its vaso-dilator effect, but a simple relatively weak vaso-constriction in its place, the depressor equivalent of the liver extract still produced at each injection a vaso-dilatation, less pronounced, indeed, than that which it initially evoked, but quite definite.

This indication of the presence in the extract of another vaso-dilator agent, the effect of which persisted when that of histamine had disappeared, was obtained in every perfusion experiment made on the leg of a cat, whether with blood or with a gum-saline solution. The nature of this other principle became clear when we further added a small dose of atropine (0.2 mg) to the perfusing blood. When the vaso-dilatation, which atropine itself produces under such conditions, had subsided, and the perfusion had again become regular and the limb-volume steady, we again compared the action of histamine with that of a depressor equivalent of the liver extract. They now produced identical effects of vaso-constriction, the additional, and previously persistent, vaso-dilator action of the extract had been abolished by the atropine, and the histamine-like effect alone remained. The probability that the other vaso-dilator substance was choline became very strong, and was increased when we found it possible to make an artificial mixture of histamine and choline, with which the effect of the liver extract at every stage of a perfusion experiment could be reproduced with great precision. The particular sample of liver extract which we were then using was matched in activity by one containing 0.1 mg of histamine and 17 mg of choline per c.c.

It will be obvious that the conditions of the perfusion experiment on the cat's leg, in which the capillary-dilator effect of histamine is at best evanescent and always relatively weak, gave an undue prominence to the arterio-dilator action of the choline-like constituent. We found, indeed, that the addition of choline to a histamine solution, in the proportion above indicated, made but a trifling addition to its depressor effect on the anæsthetised cat, though it had been so effective on the perfused vessels. This contrast is readily explained, if we suppose, as a large body of evidence entitles us to do, that the vascular tone is strongest

in the smallest peripheral vessels, including the capillaries, of the cat under ether while in the perfused organ the capillaries rapidly lose their tone so that the resistance to the flow is encountered almost entirely in the arterioles. The effects observed on the perfused vessels had made it clear however, that the vaso-dilator effects of choline and histamine should not be simply additive. The relatively weak constrictor effect of a small dose of histamine on the arteries is we have seen easily out-balanced by the vaso-dilator effect of choline on the same vessels. Even in the anaesthetised animal we must suppose that the dilator effect of histamine is the resultant of a strong peripheral dilator effect and a weak, less peripheral constrictor effect. If choline is injected simultaneously in quantity sufficient to reverse the latter so that vaso-dilatation occurs throughout the whole peripheral branching the combined effect should be greater than a sum of the two. This deduction was put to the test of experiment in the following way. Choline in small doses up to about 1 mg. causes no slowing or detectable weakening of the heart-beat, but apparently acts on the circulation as a pure vaso-dilator. Its depressor action is not qualitatively identical with that of histamine, the time-relations being somewhat different. Nevertheless it is not difficult to determine doses of the two substances which produce brief falls of the arterial pressure to identical minima. The ratio so determined varied rather widely from one experiment to another, and was by no means constant throughout one long experiment, with a shift in the incidence of vascular tone we should, indeed, expect it thus to change. It was sufficiently stable over short periods however, for the comparison we had in view. Having determined the doses producing practically identical minima—say, 0.001 mg. of histamine and 0.3 mg. of choline—we prepared solutions containing these doses in equal volumes, and mixed the two. Of this mixture we now injected a dose containing half the previously injected dose of each substance—0.0005 mg. of histamine and 0.15 mg. of choline in the instance cited. The effect was regularly greater than that of the original dose of either, whereas if the effect were simply additive the combined

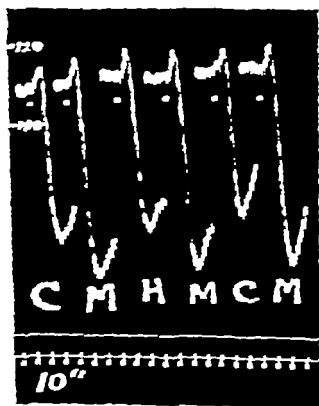


Fig. 1. Carotid blood pressure of cat under ether. Intravenous injections—at C in each case, 0.3 mg. carmine chloride; at H 0.001 mg. histamine; at M in each case, 0.0005 mg. histamine + 0.15 mg. carmine chloride.

half-doses should obviously have produced the equivalent of one whole dose of either constituent. The observation was repeated on several cats, and on one dog, always with the same result. Fig. 1 shows a typical example of this potentiating effect.

So far the evidence was entirely compatible with the supposition that the immediate, evanescent, vaso-dilator effect which we were studying was due to the presence in the extract of two substances, one closely resembling histamine in its action, and the other as closely resembling choline. Since a choline effect can be eliminated by atropine, while the rabbit under the anaesthetics usually employed is not sensitive to the vaso-dilator action of histamine, a test on this species would give further evidence as to the completeness with which these two types of action would represent that of the extract. On a rabbit anaesthetised with veronal, intravenous injection of doses from 0.12 to 1 c.c. of the liver extract caused a relatively small, but regular and definite fall of arterial pressure. 2 mg. of atropine were then injected, and a test with a small injection of acetylcholine showed that paralysis of response to this type of action was complete. A further injection of the liver extract, however, caused a smaller but still definite fall of the arterial pressure. Careful watching of the record during its production showed that the depression was accompanied, and could probably be accounted for, by a pronounced retardation of the heart-beat. As the rate returned to the normal, the blood-pressure returned to the original level. This was not an effect of the type which we were investigating. We found, further, that when the extract was purified by precipitation with basic lead acetate, it no longer slowed the heart-rate or lowered the blood-pressure in the atropinised rabbit, but produced only such a small rise of pressure as a small injection of histamine would produce, whereas this purification left the pronounced action on the arterial pressure of the cat practically unchanged. Since we were concerned to investigate only substances lowering the blood-pressure in the cat by immediate vaso-dilatation, the effects on the rabbit confirmed the view that we were dealing with associated effects of the choline and the histamine types only.

#### PHYSIOLOGICAL EFFECTS OF LUNG EXTRACTS

As above indicated, liver extract was chosen, in the first instance, for investigation, on account of the practical interest attributed to it at the time. So far as immediate vaso-dilator effect is concerned, however, we found that it did not exhibit any special intensity of action as compared with other organs. On the contrary, when different extracts, made

by identical procedures, were compared, that from the liver was among the weaker ones in action of the type which we were studying, while that from the lung stood out above all the others so far tested, as by far the most active. In general terms it may be said that, weight for weight, lung tissue yielded about 10 times as much vaso-dilator substance as the most active of the other tissues, and about 20 times as much as liver. The work of chemical separation of the liver principles, with a view to their identification, was already in hand. It was thought desirable, therefore, to apply the same processes to lung extract, so that a comparison between the initial activities and the final yields of pure principles might eventually be made.

No such complete physiological analysis of the action was made in the case of lung as in that of liver. It was sufficiently obvious that the action was predominantly of the histamine type, atropine making no sensible difference in the depressor activity of the whole extract, as determined in comparison with pure histamine on the cat under ether. We found, further, that a dose of the lung extract equivalent in depressor action to the fatal dose of histamine, killed the unanæsthetised guinea-pig, with the typical bronchial occlusion, when injected intravenously.

#### ISOLATION AND IDENTIFICATION OF CHOLINE AND HISTAMINE FROM LIVER AND LUNG

##### *Introduction*

The physiological evidence having indicated the presence of principles acting like histamine and choline, we naturally tested the possibility of separating the unknown principles by the chemical methods which are used for the isolation of those bases. Choline, which had often been obtained from tissue extracts before, we expected to obtain. It was doubtful, on the other hand, whether histamine had ever been isolated in chemical purity from material under no suspicion of bacterial contamination, and, so far as we are aware, it had never been so obtained from liver or lung. It will be seen that we have isolated it in relatively large quantities, corresponding to a high proportion of the vaso-dilator activities of the original extracts.

Before we give the details of the procedures leading to their isolation, it will be useful to mention certain points in our experience of the behaviour of these bases, when present in mixtures with other constituents of an organ extract. At the successive stages of the isolation we have met with examples of their being precipitated where they ought

to remain dissolved, remaining in solution in the presence of reagents which ought to precipitate them, failing to be extracted by solvents which ought to remove them, or disappearing under treatment to which they should be stable. A few examples may be given.

(1) At the outset of our work we had before us the fact that Best and Scott at the Connaught Laboratories of the University of Toronto, in preparing the liver extract, found that substances responsible for the depressor action here in question were largely precipitated, from solution in 80 p c alcohol, by the addition of 3 volumes of acetone. This precipitate was dissolved in water to furnish the preparation used in therapeutics, and this solution was the starting point of our own investigation. When histamine and choline are dissolved in 80 p c alcohol, in proportions yielding a much more actively depressor solution, the solution remains perfectly clear when 3 volumes of acetone are added. The conclusion might easily be drawn that the depressor action of the precipitate from the liver extract is not due to either of these bodies, but the conclusion would be wrong.

(2) If sodium carbonate in excess is added to a solution of histamine in water and the mixture evaporated to dryness, the histamine can be extracted almost quantitatively from the residue by hot chloroform. This method was extensively used by Abel and Kubota<sup>(9)</sup>, who regarded any depressor substance not so extracted as not histamine. Koessler and Hanke<sup>(15)</sup> showed, and we can confirm the observation, that from a pure solution of histamine, or one containing histidine in addition, the histamine can be separated by adding excess of caustic alkali and shaking with amyl alcohol. We found that preparations, in which a histamine-like action had been concentrated into a small bulk of relatively purified solution, failed to yield any significant amount of histamine to either of the above methods of extraction, and, further, that the proportion recognisable physiologically in the alkaline residue was in some instances only a small fraction of that originally present. We were at first inclined to regard this as definite evidence that the depressor substance was something other than histamine, until we found that histamine itself, added in known quantity to a sample of such a solution, was not extracted by these methods in larger proportion than the natural depressor agent, and that nevertheless it disappeared to varying extents in the alkaline mixtures from which its extraction was attempted. Other methods for extracting the depressor substance had to be found, and when it was extracted it proved to be wholly histamine.

(3) In connection with the experiment on lung (see below) it was found that a small, but appreciable amount of depressor material escaped precipitation with phosphotungstic acid. At first we were inclined to believe that this indicated the presence of active substances other than histamine and choline. It was discovered, however, that this assumption was false, and that the precipitation of the histamine and choline (and other bases) had been incomplete owing to the large amount of sodium acetate in the solution.

(4) The fact that histamine added to an organ extract disappears, as judged by the physiological test, on boiling with alkali while pure histamine solutions are stable under such conditions, was observed by Dale and Dudley (16), when investigating pituitary extracts. In our early experiments with liver extract, the apparent disappearance of depressor activity on boiling with alkali might have suggested that it could not be due to histamine. Previous experience warned us against drawing such a conclusion, which would certainly have been wrong.

(5) Depressor activity of the histamine type has been concentrated in solutions which failed to give the Pauly reaction, which was strongly developed by equidepressor solutions of pure histamine. Again we were misled, until we had tested a sample of the concentrate to which histamine had been added, and again failed to obtain the colour reaction. In some instances a positive reaction was obtained when the diazobenzenesulphonic acid was added first to the solution to be tested and the mixture was then made alkaline with sodium carbonate solution, whilst addition of sodium carbonate first and diazo-reagent last to the same solution had a negative result. In other histamine-containing mixtures this behaviour to the order in which the reagents were added was reversed, and finally, in other cases it was impossible to produce a typical Pauly reaction in solutions known to contain histamine, no matter in what order the reagents were used. We have been surprised and impressed by the unreliability of the Pauly reaction in the presence of other substances, and are very doubtful as to the value of quantitative methods based on it.

The literature, dealing with the depressor substances of tissue extracts, contains many examples of the deduction, from the apparent physical or chemical properties of the agent in a particular case, that it cannot be histamine. Having been ready to draw such conclusions ourselves, we have become by experience ever more doubtful of their soundness. In the case of histamine especially, and of choline in minor degree, when identity of physiological action has been proved, the

evidence of chemical difference needs the closest scrutiny and control, before it can be accepted as decisive

#### METHODS EMPLOYED FOR THE ISOLATION OF CHOLINE AND HISTAMINE

The methods which led to the successful isolation of these two substances followed the lines of those in general use for the separation of bases. After preliminary purification with basic lead acetate, which occasioned only such loss of activity as was inevitable in dealing with bulky precipitates, the bases were precipitated as phosphotungstates, which contained practically the whole of the activity. From this precipitate the bases were regenerated and fractionated by the silver method of Kossel and Kutscher. Choline was isolated as the mercurichloride from the "lysine" fraction, and in one experiment by direct treatment of the neutralised solution of the bases, liberated from the phosphotungstate precipitate, with mercuric chloride.

As Barger and Dale had already observed, in isolating histamine from ergot(17), the histamine-like activity did not all come down in the "histidine" fraction but was spread over this and the "arginine" fraction. These two fractions were accordingly precipitated together, thus a solution containing both bases and basic acids was obtained, and success or failure in obtaining the histamine from this depended on the degree to which the unwanted constituents of the mixture were removed, before picric acid was applied to precipitate the histamine itself. It was frequently found possible to obtain further quantities of pure histamine picrate from mother liquors, which refused to yield more of the material directly, by removing the picric acid and submitting the residue to the process of fractionation again.

The first task was to devise a method for the separation of the bases from the basic acids of the "histidine-arginine" fraction. As stated above, the properties of histamine are so modified by the presence of other substances, that chloroform extraction of the residue obtained by evaporating the solution to dryness, after adding excess of sodium carbonate, was unsuccessful, and the same applies to many other modifications, based on the general principle of anchoring the acids as salts and then extracting the free bases with a solvent, in which the former are insoluble.

The method which proved, in our hands, most satisfactory was to grind up the concentrated solution with a large excess of baryta, sometimes with the addition of a little plaster of Paris, and, after thoroughly





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trated to 100 c c *in vacuo* and acidified with a small quantity of acetic acid. Hot mercuric chloride solution was added until precipitation was complete. After filtration sodium acetate was added until no further precipitate formed. The combined precipitates were dissolved in boiling water from which, after filtering off a small insoluble residue, 260 gm. (= 17.5 gm. base) of pure *choline mercurichloride* (M P 250°) were obtained. The identification was assured by preparing the picrate and chloroaurate from this salt. The properties of these salts coincided with those of choline, the picrate melted at 242–4° and the chloroaurate at 266–9° (with decomposition).

*Analysis of chloroaurate* 0.1938 gm. gave 0.0855 gm. Au

Au found 44.12 p c

Au calculated for  $C_5H_{14}ON$ ,  $AuCl_4$  44.47 p c

### *Lung extract*

*Histamine* 10 kg. fresh ox lung were minced and extracted with alcohol as described for liver. The acetone precipitation was omitted. The extract was concentrated *in vacuo* to a small bulk, shaken out with ether to remove fat and finally reduced *in vacuo* to a volume of 2 l. It was then purified and fractionated in the same manner as the liver extract.

The physiological activity of the "histidine-arginine" fraction corresponded to that of 400 mg. histamine. Alcoholic extraction of the baryta-treated material recovered activity equivalent to 300 mg. histamine. From this extract 1.288 gm. pure histamine picrate was obtained, and on grinding up the mass in the Soxhlet thimble and re-extracting with alcohol a further quantity of 0.146 gm. histamine dipicrate was obtained. In all therefore 1.434 gm. pure histamine dipicrate (= 287 mg. base) was obtained.

*Analysis* 0.0406 gm. picrate gave 0.0773 gm. nitron picrate

Picric acid found 80.58 p c

Picric acid calculated for  $C_5H_9N_3$  ( $C_6H_3O_7N_3$ )<sub>2</sub> 80.48 p c

The more soluble fractions from the recrystallisation of the histamine dipicrate were freed from picric acid, and, after adding a large excess of sodium carbonate, dried and extracted with hot chloroform. A small amount of depressor substance was extracted, but it was not possible to isolate the histamine from this in a state of purity. This alkaline residue was then extracted with alcohol and 0.115 gm. of a non-depressor picrate (M P 199°) was obtained from the extract, which was identified as methylguanidine picrate.

*Histamine* The precipitate was suspended in dilute sulphuric acid, a sufficient quantity being taken to render the liquid just acid to Congo red.  $H_2S$  was then passed through the liquid which, after removal of silver sulphide and barium sulphate by filtration, was neutralised with baryta and concentrated *in vacuo* to 25 c c. It gave a strong Pauly reaction, and, tested physiologically, contained depressor activity equivalent to 240 mg of histamine.

25 gm crystalline barium hydroxide were added to the solution, the mass was ground up and then thoroughly dried in a vacuum desiccator over sulphuric acid. It was then ground to a fine powder and extracted for 3 hours with absolute alcohol in a Soxhlet apparatus. The extract was diluted with water and neutralised with sulphuric acid, some baryta had been extracted and this was precipitated as sulphate. After removing the alcohol *in vacuo* the barium sulphate was filtered off. Physiological assay of the solution showed that it contained depressor activity equivalent to 75 mg histamine. The residue in the thimble had formed a hard non-porous mass. It was therefore ground up afresh with a little plaster of Paris and again extracted, this procedure was repeated three times. The combined extracts then displayed physiological activity corresponding to 122 mg histamine. They were concentrated *in vacuo* to 25 c c and treated with sodium picrate. 608 mg of a crystalline picrate were obtained (M P with decomposition,  $220^\circ$ ). After recrystallising twice from water 431 mg of pure *histamine dipicrate* (= 86 mg base) were obtained (M P with decomposition,  $241^\circ$ ). It was identified by analysis and comparison with a specimen of histamine dipicrate, mixed melting point determinations showed no depression, whilst crystalline form and physiological activity were identical.

*Analysis* 0.0410 gm picrate gave 0.0779 gm nitron picrate

Picric acid found 80.41 p c

Picric acid calculated for  $C_5H_9N_3 (C_6H_3O_7N_3)_2$  80.48 p c

No attempt was made to procure the maximum possible yield of pure histamine dipicrate from this batch by reworking the mother liquors. In another experiment, which is summarised in the Table on p. 412, the amount of histamine dipicrate actually isolated was increased by slightly more than 35 p c by exhaustive treatment of the residues.

*Choline* From the "lysine" fraction (*i.e.* the filtrate from the precipitation with silver nitrate and baryta) silver and barium were removed in the usual manner and the bases reprecipitated as phosphotungstates. The precipitate was decomposed with baryta in the cold, excess Ba removed as sulphate and the neutral liquid was then concen-

It is obvious therefore that the factor by which the result of the actual test must be multiplied is very large, and it is unreasonable to expect a high degree of accuracy. A comparison of the figures under "basic lead acetate" and "phosphotungstic acid," therefore, merely indicates that the histamine-like activity is quantitatively precipitable by the latter reagent, and recoverable without appreciable loss from the precipitate.

Throughout the fractionations all final mother liquors and discarded precipitates were tested physiologically, and in no instance was any evidence obtained which indicated the presence of any depressor substances other than histamine and choline.

It is interesting to note that, in the experiment with 66 kg. ox liver, the proportions of choline and histamine actually isolated in chemically pure condition are very close to the ratio (17 mg. choline to 0.1 mg. histamine) determined by physiological assay of the extract by Best and Dale before the chemical investigation was commenced. It appears therefore that the proportional loss involved in the method of fractionation is approximately the same in the case of each base.

An outstanding quantitative fact, which has been revealed by this investigation, is the very high content of histamine in lung as compared with that in liver, and in other tissues on which work is proceeding. It will be seen that, averaging the above figures, 1.58 mg. pure histamine has been isolated per kg. of liver, whilst 27.64 mg. were obtained per kg. of lung. There is a reasonably good correspondence between the physiological observations on the relative depressor effects of the original extracts from the two tissues and the yields of histamine obtained from them by chemical fractionation.

#### DISCUSSION

It is desirable, perhaps, still further to emphasise the limitations of our problem. We were not directly concerned with the question whether extracts prepared from liver, or other organs, produce a lowering of pathologically high blood-pressure, delayed in onset and long continued in effect. We were concerned with the immediate and evanescent depressor effects shown by such extracts, when injected intravenously into a cat or dog. We were aware, indeed, that this immediate action was in use for the assay of one type of liver extract, and had appeared to give an index of its activity in producing a prolonged therapeutic action. If the existence of this latter should be established by others, it may become desirable to consider whether it may be due to the slow liberation of histamine from association with the more complex constituents of a crude

Two curious and unexpected facts were encountered in the investigation of the lung extract. The lysine fraction contained no substances precipitable in the usual way by phosphotungstic acid and the filtrate from the first precipitation of the bases as phosphotungstates, when tested physiologically, contained depressor material of activity equivalent to 40 mg histamine.

As no such quantity had escaped precipitation in any of the previous experiments on lung and liver extracts the matter was investigated. It was found that the large amount of sodium acetate<sup>1</sup> in the solution was responsible for the failure of phosphotungstic acid to precipitate completely the histamine and other bases present, for, after most of this salt had been removed and the remainder converted into sulphate, the addition of phosphotungstic acid produced a copious precipitate from which choline was isolated and a mixture of the picrates of histamine and creatinine obtained. Methylguanidine too was isolated from this precipitate in considerable quantity as the picrate. No doubt remained, therefore, that the depressor activity which at first had escaped precipitation by phosphotungstic acid was, in fact, due to choline and histamine.

Appended is a table showing the physiological assays at each stage in the separation of histamine, in the two experiments given in detail above, and in two others conducted on similar lines. In the experiment with 72 kg of ox liver, 97 mg histamine (as dipicrate) were isolated directly. By reworking the residues a further quantity of 178 mg histamine dipicrate (= 36 mg histamine) were recovered.

The activities are expressed as milligrams of histamine.

TABLE

	Original extract	Basic lead acetate	Phospho- tungstic acid	Silver fraction	Alkaline extraction	Pure histamine (isolated as dipicrate)
Ox liver (66 kg)	—	250	320	240	122	86
Ox liver (72 kg)	390	195(?)	280	204	140	133
Ox lung (500 grm)	22	17	16	12.5	10	3.2
Ox lung (10 kg)	750	530	460	400	300	287

It will be observed that in the experiments with liver extracts the physiological evaluation of the amounts of histamine obtained after decomposition of the phosphotungstates is somewhat higher than that after the previous stage of the fractionation. The physiological assay is based on the comparison of very small volumes (0.003–0.03 cc) of the solutions in question with amounts of histamine of the order of 0.003 mg.

<sup>1</sup> Most of the sodium was introduced by using a solution of sodium phosphotungstate in 5%  $H_2SO_4$  for the precipitation of the bases.

would probably have shown no higher proportion to the quantity added than the histamine which we actually isolated bore to the naturally occurring depressor agent. When it is also remembered that choline made some contribution to the "histamine equivalent" determined in the earlier stages of the purification, and that it would increase the apparent amount of "histamine" to a degree out of proportion to its own separate activity, we think we may fairly claim to have established a strong probability that the whole of the histamine-like activity of the lung extract is due to histamine itself.

In the case of the liver extract, the isolation of histamine was rendered more difficult by the fact that it was present in much smaller proportion, in a solution containing a much larger quantity of inactive constituents. It is, further, not without significance that the chemical work on the lung extract was carried out later, and therefore with the aid of additional experience. We have little doubt that we could now isolate a larger proportion of the histamine-like constituent of liver extract as histamine itself. Although, therefore, the actual quantity isolated was somewhat less in proportion to the physiological equivalent originally determined than in the case of the lung extract, we think it probable that in the liver extract also, the histamine-like activity was due to the presence of histamine itself. The position is very similar in the case of choline. We did not follow it so systematically by physiological assay through the different stages. Since, however, the proportion between the quantities of choline and histamine finally isolated was roughly the same as the proportion determined from the physiological activities of the original extract, the loss was evidently of the same order in the two cases. We find no good reason for supposing that the excess of the choline-like action originally present, over that of the choline isolated in chemical purity, was due to any other substance than choline itself.

(2) It is of interest to enquire whether the bases are present in the tissues as such, or separated from some combination in the process of extraction. The problem in the case of choline is no new one. Choline has previously been obtained from many tissues, and much has been written in support of, or in opposition to, its claims to be the depressor substance present in such extracts. The much greater activity of histamine gives a proportionately greater interest to the question of its presence as such in the tissues. The method of extraction which we have used is of the simplest possible kind. The perfectly fresh tissue has been disintegrated as rapidly as possible and extracted immediately with cold alcohol. Some small-scale experiments, in which only a

tissue extract—a possibility not incompatible with some of our own observations on the curious behaviour of that base. We are not, however, here concerned with such possibilities. Further, it was no part of our purpose to account for all the falls of arterial blood-pressure which have been described as following the injection of various crude extracts and suspensions—effects in some of which intravascular precipitation, fibrin formation, agglutination of corpuscles, or endothelial injury might conceivably be concerned. Our object was to discover the nature of the depressor substances which occur in extracts from practically all tissues, are soluble in water and strong alcohol, and produce an effect in which a predominantly histamine-like action can be recognised. At the present stage our evidence is further limited to extracts of liver and lung. In the case of the liver extracts used by us, in the first instance, and later in that of the lung extract, though there to a much smaller degree, we found that the histamine-like depressor effect was complicated and reinforced by a choline-like action. In neither case did we detect any important depressor action which did not correspond to one of these. From these extracts we have isolated histamine and choline in quantities sufficient for complete chemical identification. A series of questions arises with regard to this identification, some of which can be met on the evidence before us, while some suggest problems rather for further investigation.

(1) We must consider whether the quantities of pure bases isolated warrant the supposition that the whole of the effect which we were studying was accounted for by their presence. In the case of the lung extract we consider that the figures which we have given render such a supposition reasonable. It is true that the original estimate, on the crude extract, indicated the presence of a physiological equivalent of 750 mg of histamine, and that slightly less than 300 mg of that base were finally isolated as the pure dipicrate. It will be seen, however, that there was no exceptional loss at any one stage of the long process of purification, such as to indicate that any part of the activity was due to much more complex substances. When we tested the purification by basic lead, or the precipitation by phosphotungstic acid or by silver, on a small scale, there was no indication of any definite loss, the activity was wholly in the filtrate, or wholly in the precipitate. On the large scale the mere bulk of the materials and the physical properties of some of the precipitates made quantitative recovery a practical impossibility, and we did not, in fact, expect to obtain it. We were left with the clear impression that, if we had actually added histamine to a large bulk of such an extract, and attempted to isolate it by the same procedure, the yield

tissues, and, in the case of the lung especially, a surprisingly abundant one. These facts seem to justify the expectation that this potent base will be found widely distributed in the tissues, and to reinforce the suggestion that it must have some important function in the control and adjustment of the circulation through the small blood vessels.

## SUMMARY

Histamine and choline have been isolated from alcoholic extracts of fresh liver and lung, in quantities sufficient to account for the immediate vaso dilator activities of those extracts. Histamine is responsible for the greater part of this activity, and is present in remarkably large amount in the extract from lung. The physiological significance of its occurrence is discussed.

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physiological estimate has been made, have been carried out with even greater care. The chest of a living animal was opened under anæsthesia, a lung was removed, dropped straight into alcohol cooled with solid  $\text{CO}_2$ , and thus frozen immediately, it was then minced while frozen and returned to the cold alcohol. The extract thus obtained showed a histamine-like activity of the same order as that obtained without these extreme precautions, and as great as that of a similar extract made from the corresponding lung, removed an hour after the animal's death. There is good reason, therefore, to believe that the histamine is present as such, at least as soon as the cells are killed by contact with the extracting alcohol. The evidence, indeed, of its presence in the cells during life may be said to be as good as that of the presence of lactic acid in a living muscle, or even rather better, since we have found no evidence that the proportion of free histamine increases in the period immediately following the death of the animal.

(3) Histamine being either present as such in the living cell, or released therefrom at the moment of death, questions of great interest arise as to the manner in which it is held by the protoplasm during life, and prevented from producing its intense physiological action. It may be there only potentially, in the form of some inactive precursor. On the other hand, it is conceivable that histamine is present as such in the cell interior, being prevented from leaving it so long as the cell membrane is physiologically intact, and that it produces its action only if some stimulus or injury causes its escape into the extra-cellular fluids. On these points we have no evidence, and it may be very difficult to obtain it.

(4) The presence of so much histamine in a tissue, as can be extracted from the lung, can hardly be without physiological significance. 100 gm of lung tissue—say, the lungs of a dog—may be expected to contain as much as 7 or 8 mg of the base—a quantity which, if suddenly released into the circulation of the animal, would have a profound, shock-like action. Merely to mention two possibilities, which can only be considered in the light of further and more direct evidence, it is conceivable either that the lung acts as an organ of internal secretion with respect to histamine, or, on the other hand, that it merely captures histamine which has escaped into the venous blood from more vigorously metabolic tissues, and holds it pending its final disposal. In the latter case the blood would not only be oxygenated, but also physiologically purified, during its passage through the lung.

Here, however, we are merely concerned with the demonstration of the fact that histamine itself is the main depressor constituent of certain

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**Changes in the pituitary after section of the right vagus**

By E C EAVES and G A CLARK (*Preliminary communication*)

One of us (G A C) in a paper now in the press has suggested the possibility of tonic inhibitory fibres in the right vagus to the islets of Langerhans

In examining the pituitary glands from rabbits at varying times after section of the right vagus we were struck by the large size of the glands in many cases, and were led to investigate the histological appearances. Up to the present the appearances found have not been compared with those of strict control animals, but the difference between the pituitary after vagotomy and that in normal rabbits is so striking and suggestive that we thought it worthy of description.

One of us (E C E) had previously found changes in size and histological structure of the pituitary after insulin injections (A description is given in the *Proceedings of the Physiological Society* in this number). There is a similarity between the appearances seen in the pituitaries of the insulin-injected rabbits and those of the vagotomy rabbits.

In the pars intermedia the cells seem large and blurred compared with those in the normal pituitary, and they sometimes merge into a mass of secretion. It is not possible at present to indicate differences in the pars intermedia at different stages after vagotomy. A few weeks after this operation the cells of the pars anterior exhibit increased eosinophilia and become large and confluent. At a later period—21 weeks after vagotomy—the eosinophilia is confined to patches of hypertrophied cells which are very coarsely granular. Many of these show vacuoles in their interior. Surrounding these groups of hypertrophied eosinophils are vacuolated chromophobe cells. The appearance is suggestive of the hypertrophied eosinophils discharging their secretion and becoming converted into chromophobe cells.

It seems possible that the histological changes are brought about by increased insulin (which would occur if the tonic inhibitory influence was removed from the islets of Langerhans by section of the vagus).

The expenses of this research have been defrayed by a grant from the Medical Research Council.



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**The effect of pituitrin on blood sugar** By C D TINGLE and  
C G IMRIE (*Preliminary Communication*)

In a case of diabetes insipidus with rigidity following epidemic encephalitis, the effect of pituitrin on the blood sugar was studied at half-hour intervals, under the following conditions

(1) On three occasions 0.5 c.c. pituitrin was given subcutaneously. The results of half-hourly estimations of blood sugar are shown in P 1 to P 3

P 1	P 2	P 3	G 1	G 2	G 3
083	075	085	080	085	085
215	187	190	131	147	134
124	117	114	102	106	086
102	088	090	092	085	070
083	076	070	072	065	078
—	—	084	077	—	—

For comparison with these, the effect of 50 grm. glucose given to the same subject on three different occasions, is shown in G 1 to G 3. It is to be noted that

- (a) the effect of 0.5 c.c. pituitrin is greater than that of 50 grm. of glucose,
- (b) the fasting level of the blood sugar in this patient is below the average and the effect of glucose decidedly so

Also for the sake of comparison with these figures, the following results of the injection of 0.5 c.c. pituitrin into each of three other subjects, the first two of them normal, the third a case of post-encephalitic rigidity who gave a normal response to glucose, are here given

(1)	(2)	(3)
101	092	095
174	186	172
114	091	118
086	085	085
068	081	071
081	096	091

(2) On three occasions 0.5 c.c. pituitrin and 50 grm. glucose were given simultaneously

081	081	080
134	124	129
213	228	223
168	116	161
079	096	076
066	079	—

In the first half-hour the blood sugar rises less than with pituitrin alone and no more than with glucose alone, in the second half-hour a much larger rise occurs, which is similar to that given in the first half-hour by pituitrin alone. There is no direct summation of the two effects

(3) If 0.5 c.c. pituitrin is given half an hour after 50 gm glucose, an effect is obtained which is almost the same as in (2), when they are given simultaneously

090	089
134	125
202	209
136	143
093	—
075	087
086	—

(4) On the other hand when 0.5 c.c. pituitrin was followed half an hour later by 50 gm glucose, the result on two occasions was

087	077
169	176
217	205
161	148
085	091
058	073
068	090

Here when pituitrin acts for half an hour before glucose, the pituitrin effect comes on at once and the glucose effect is superadded afterwards

These observations suggested that when the blood sugar is raised the effect of pituitrin is delayed until it begins to fall. This suggested the study of the effect of pituitrin in the hyperglycaemia of diabetes mellitus. The results of injecting 0.5 c.c. pituitrin in three diabetic subjects are given

193	201	313
195	199	239
103	155	267
135	164	304
167	177	312
193	199	—

In each instance a lowering of the blood sugar occurred. In the first two with blood sugar about 0.2 p.c., the fall did not take place till the second half-hour, in the third with blood sugar 0.313 p.c., it occurred in the first half-hour. At no time did it rise as in normal subjects.

The lowering of the blood sugar in these patients is remarkable, as they were diabetic, the production of insulin was defective.

### The action of insulin on liver diastase By W SMITH

Langfeldt, using liver diastase working in a phosphate buffer, found that the optimum  $p_H$  was 6.23. He stated that this was shifted to the alkaline side  $p_H$  7.73 by the action of adrenaline. Since adrenaline and insulin are in some respects antagonistic it seemed of interest to study the action of the enzyme in this case.

The diastase was prepared according to the method of Wiechowski. The optimum was found to be  $p_H$  6.8. This value was also obtained from pancreatic diastase prepared from the sheep, that from the ox being a little more acid,  $p_H$  6.5. Experiments did not show any marked shift when adrenaline was added in the concentrations used by Langfeldt. This was tried several times with different samples of commercial adrenaline hydrochloride.

The earlier experiments with insulin showed a marked inhibition of the diastatic action, but no change in the optimum reaction. This inhibition was constant, using the same sample of insulin. When other samples were used the effect noted was either, not so marked, or, entirely absent. It seems probable that this is due to another substance being present causing the inhibition. In this connection one may refer to the action of insulin on the hæmoglobin concentration of rabbits, which was found to be due to some other factor. One is forced to conclude that insulin does not inhibit liver diastase, but that if inhibition takes place, it is due to the presence of another factor. As a rule it was found that "crude" insulin (the fraction precipitated by 93% alcohol in the Collip preparation) does contain this inhibitor.

### The nitrogenous metabolism in post-encephalitic rigidity

By M. HIRST and C. G. IMRIE (*Preliminary Communication*)

It has been suggested<sup>(1)</sup> that the low rate of uric acid excretion at night and the high rate in the forenoon are related to varying degrees of activity of muscular tone. It seemed possible that additional evidence for this hypothesis might be furnished by a study of the excretion of uric acid in subjects exhibiting marked rigidity following epidemic encephalitis. The subjects were put on a diet free from nucleo-protein and creatine for six days or more before the analyses were begun. Urine was collected at 7 a.m., noon, and at 5 and 9 p.m. daily. Uric acid was estimated by Folin Shaffer's method.

The following table shows the results for uric acid in mg. per hour

Name	No. of days	7 a.m. to noon	Noon to 5 p.m.	5 to 9 p.m.	9 p.m. to 7 a.m.	Total for 24 hours
E. L. K. (normal)	21	23.3	20.7	16.6	12.8	429
Herd	8	21.45	19.76	14.1	12.5	392.8
Wilkins	10	22.75	20.2	15.3	12.6	400.6
Newton	5	22.3	19.9	16.1	7.8	352
Henderson	3	27.1	18.7	19.6	16.8	475.2
Fern	8	15.3	13.6	12.0	7.9	266

Wilkins (rigid) has the same diurnal tide as Herd (not rigid) and E. L. K. (normal).<sup>(1)</sup> Newton (rigid) is similar but that the output at

night is only 35 p c of the morning output as compared with 55 p c in the normal Henderson (very rigid) shows a relatively high output at night (62 p c of the morning output), and a high total output. Fern (rigid, female) excretes at night 52 p c of the amount excreted in the morning, and shows no considerable difference from the normal figures.

For comparison with these results, similar analyses were carried out while two of the subjects were treated with hyoscine. In both these cases voluntary movements were facilitated as a result of this treatment though the condition did not become normal.

Name	No of days	7 a.m. to noon	Noon to 5 p.m.	5 to 9 p.m.	9 p.m. to 7 a.m.	Total for 24 hours
Henderson	3	27.1	18.7	19.6	16.8	475.2
After hyoscine for 3 days	3	26.1	27.7	21.6	19.6	552
After hyoscine for 5 weeks	6	20.9	17.8	13	13	376
Wilkins						
Before hyoscine	10	22.75	20.2	16.6	12.8	401
During hyoscine for 7 days	7	20.6	14.6	21.2	11.1	395

The effect of hyoscine on Henderson was to diminish the output of uric acid, the daily output falling about 20 p c, in the first three days the output rose about 20 p c, after this short rise the fall was maintained till the end of the investigation (6 weeks). It is true that during the time when his output was low, his diet contained less nitrogen than during the time when it was high. But this change in the nitrogen intake, though it would tend to diminish the absolute amount of uric acid excreted, would cause the relative amount (uric acid nitrogen/total nitrogen p c) to increase (2). The fall of uric acid output following hyoscine treatment in this patient was sufficient to efface such a relative increase.

On Wilkins, the effect of hyoscine was not to alter the daily output but to alter the time of day at which the uric acid was excreted. This subject slept daily for four hours after the hyoscine (between 12 and 5) and during this period of the day the uric acid output was conspicuously diminished, rising again in the period from 5 to 9 p.m. Henderson never slept during the daytime in the course of the experiments.

Evidence exists for a connection between muscular functions and the output of creatinine and creatine, e.g. (3), (4), (5). The creatinine excretion was determined by Folin's method at the same time as the uric acid. This seems to present no features differing from those observed in normal subjects, the daily output ranging between 1.3 and 1.5 gm in the male cases and 0.88 gm in the female case.

Creatine estimations however were also carried out. Herd who was not rigid was found, like normal subjects, to excrete none at all. The



four subjects who exhibited rigidity all excreted creatine as well as creatinine. The figures for the different periods of the day, as well as for the 24 hours, are shown in the following table

Name	No of days	7 a m to noon	Noon to 5 p m	5 to 9 p m	9 p m to 7 a m	Total for 24 hours
Herd	8	0	0	0	0	0
Wilkins	10	4 6	5 3	3 2	1 9	80 4
Newton	5	2 9	3 9	3 8	0	50
Henderson	3	5	4 9	6 6	3 1	107
Fern	8	12	9 2	10 2	6 9	215

As a result of this investigation it appears

(1) With regard to uric acid, that while in Henderson the output was high and was notably reduced by hyoscine and in Wilkins it was reduced during the sleep brought on by hyoscine, no close and constant relationship between this type of rigidity and uric acid excretion has been established

(2) That in addition to the normal output of creatinine, a degree of creatinuria has been found in all the four cases examined

- (1) Cathcart, Kennaway and Leathes Quarterly Journal of Medicine, 1 No 4, p 416 1908
- (2) Folin Amer Journ. of Physiol 13 p 66 1905
- (3) Pekelharing and van Hoogenhuyze Z physiol Chem 49 p 395 1910
- (4) Powis and Raper Bio chem Journ 10 p 363 1916
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### **A note on the action of alcohol on the perfused isolated rabbit heart** By M H MacKEITH

In the course of a series of experiments undertaken with another object in view, it was found that variation of the temperature of the perfusing fluid modified considerably the effect of Alcohol in lowering the contraction-height. In all cases, the effect was most marked in the neighbourhood of 40° C, and decreased with fall of temperature as long as the heart continued to beat regularly (*i e* down to about 15° C). It is worthy of note that a heart, which had been stopped in diastole by perfusion with 1/200 Alcohol at 40° C, could be restored to a regular and powerful beat by continued perfusion with the *same* strength of Alcohol, if the temperature of the perfusion fluid were lowered to 20° C, and that, if the temperature were then raised again, the heart would continue to beat, though with some diminution of amplitude, until the temperature again reached the vicinity of 40° C, when sudden arrest occurred. In the presence of Alcohol, the *heart-rate* was affected by changes of temperature in the usual

way, i.e. the rate increased *pari passu* with rise of temperature, except when, at the higher temperatures, definite disorders of conduction appeared

Although, by lowering the temperature to the vicinity of 20° C, the depressant action of Alcohol could be almost, if not entirely, abolished, in no case was definite evidence of a stimulant action of Alcohol on the isolated heart observed. The above influence of temperature was manifested at all concentrations from 1:50 to 1:1250, after varying times of previous perfusion, and in the absence of glucose from the perfusing fluid, as well as in its presence. It is also worthy of note that the tolerance of Alcohol varied very widely from one individual heart to another.

### Changes in the pituitary after repeated injections of insulin

By ELIZABETH C. EAVES (*Preliminary communication*)

The original object of this investigation was to ascertain whether a condition resembling hypopituitarism could be produced in rabbits by repeated injections of insulin. This was suggested by the appearance presented by young patients treated for diabetes with insulin being somewhat similar to that in dystrophia adiposo genitalis. This is noticeable in the photographs of children treated by Geyelin<sup>(1)</sup> and has been commented on by Allen and Sherrill<sup>(2)</sup>.

Young rabbits were given four to five injections per week of insulin, the dose varying from 2 to 6 units, and were thus treated for periods varying from 8 days to 8 weeks. They were then killed, the ductless glands weighed and compared with those of normal animals of the same age.

In an adult animal the size of the pituitary was found to be independent of body weight within certain limits. A more important factor was the size of the thyroid, the pituitary being usually large when the thyroid was small and *vice versa*.

In 14 normal rabbits the average weight of the pituitary was found to be 21.8 mg.—the variations being from 18 to 26 mg. In five insulin-injected rabbits the average weight of the pituitary was 30.4 mg., the variations being from 23 to 36 mg. Histological examination indicated that the pituitaries were in a hyper-active condition compared to those from normal animals—changes being observed both in the *pars intermedia* and *pars anterior*. In the *pars intermedia* there was an enlargement and blurring of the cells, which sometimes merged into a mass of secretion. In the *pars anterior* the number of eosinophils appeared increased,

the cells were larger than normal and confluent. The latter changes were most evident in animals which had had numerous injections. There was some similarity between the appearance of the pars anterior in these animals and in one in which a thyroidectomy had been performed. Further investigations with much larger doses of insulin are now being carried out.

(1) Geyelin, H. R. *Journ. of Metabolic Research*, 2 p. 768 1922

(2) Allen, F. H. and Sherrill, J. W. *Ibid.* 2 p. 960 1922

The expenses of the research were defrayed by a grant from the Medical Research Council.

### **The antagonism of pituitrin and insulin** By G. A. CLARK (*Preliminary communication*)

It is a well-recognised fact that injection of extracts of the posterior lobe of the pituitary can prevent or diminish the hypoglycæmic response to insulin, the mode of action of pituitrin in this antagonism, however, is not known. Lawrence and Hewlett<sup>(1)</sup> suggest that "pituitrin counteracts insulin by mobilising sugar from the glycogen of the liver or muscles or both" and base this conclusion on their evidence indicating that previous injection of ergotamine abolishes the antagonism. As pituitrin reduces adrenaline hyperglycæmia it seemed improbable that it would influence the blood-sugar by sympathetic stimulation and it was decided to repeat Lawrence and Hewlett's experiments. In each of four rabbits the normal blood-sugar response to (1) insulin, and (2) pituitrin was determined after intravenous injection of ergotamine tartrate, in no case was the usual hyperglycæmic reaction to pituitrin prevented. After 2 or 3 days insulin was given after ergotamine and pituitrin injected either with the insulin or at some time during the resulting hypoglycæmia. The samples and doses of insulin and pituitrin were the same as those previously used in the animal under experiment. It will be seen from the results below that in every case there was definite evidence of the antagonistic action of pituitrin.

Rabbit 1 3 mg. ergotamine 1 hour previous.								
	Hours	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
Insulin		108	084	059	067	074	081	086
Pituitrin		103	161	141	113	109	103	—
Insulin and pituitrin together		112	137	106	107	105	101	—
Rabbit 2 6 mg. ergotamine 2 hours previous								
Insulin		105	084	062	053	050	—	—
Pituitrin		106	169	125	110	109	—	—
Insulin followed by pituitrin $\frac{1}{2}$ hour later		110	086	131	122	098	100	—

## Rabbit 3 5 mg ergotamine 2 hours previous.

Insulin	114	106	070	054	051	058	—	—
Pituitrin	113	170	234	174	136	121	—	—
Insulin followed by pituitrin 1 hour later	112	093	067	078	071	094	115	—

## Rabbit 4 5 mg ergotamine 2 hours previous

Insulin	126	096	069	055	049	045	—	—
Pituitrin	105	161	201	129	098	—	—	—
Insulin followed by pituitrin 1 hour later	103	081	062	070	078	071	092	—

Further evidence that pituitrin does not act by way of the sympathetic system is given by two experiments on cats under amvital anaesthesia. In both animals the suprarenal vessels were tied while in one the hepatic artery and portal vein were dissected free from nerves and all other structures going to or coming from the liver were tied. In both cases injection of pituitrin produced a rise in blood-sugar similar to that given in a control animal under amvital

Hours	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$
Control	100	176	135	095	090	091	—	—
No 1	126	165	176	122	084	074	083	072
No 2	113	182	140	107	103	093	—	—

(1) Lawrence and Hewlett B.M.J. p 1001 May 30, 1925

## A conception of the mechanism of normal blood clotting

By C. A. MILLS, introduced by E. H. STARLING

Normal plasma owes its stability to the lack of dissociation of its clotting constituents. Although prothrombin, cephalin, antithrombin, and fibrinogen are present, they cannot react until the molecular unions holding them are broken by some dissociating agent. Contact with dirt, glass, metal, water or injured tissue may bring about this dissociation, but in a wound it is always the last named factor that acts to initiate the clotting.

Tissue fibrinogen (Wooldridge) the active agent in tissue juices, is a protein-cephalin compound capable of uniting directly with blood fibrinogen through calcium, giving a typical clot. It is this substance in a wound which starts the dissociation of the molecular unions by its combination with some of the blood fibrinogen. This allows the liberated prothrombin and cephalin to combine through calcium, giving thrombin which at once unites with more blood fibrinogen to form fibrin. For

every molecule of fibrinogen removed as fibrin, there are about eight molecules of prothrombin and cephalin liberated to form thrombin. It is this ratio of prothrombin to fibrinogen in the plasma that determines the cumulative character of the clotting process. Were the ratio 1/1, the reaction would follow a straight line slope instead of a parabolic curve. After complete clotting, the excess thrombin is disintegrated by the antithrombin in the serum, with some regeneration of prothrombin, the antithrombin acting through its high cephalin absorbing capacity.









PROCEEDINGS  
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**Release of vaso-dilator substances in injuries of the skin<sup>1</sup>**  
By T LEWIS and I M HARMER (*Preliminary communication*)

Observations upon the reaction of the blood vessels of the human skin to mechanical stimulation, such as firm stroking with a blunt point, have provided what has seemed to be clear evidence that this reaction is due to the release in the skin of minute quantities of a substance, histamine-like in nature and acting locally on the endothelial vessels and sensory nerve endings (Lewis and Grant, "Heart," 1924, 11 209). Probably because the quantity of substance released is extremely small, efforts to recover it from the skin, and to demonstrate its presence by physiological tests, have so far failed.

Recently we have adopted a different procedure. We select subjects in whom the skin reacts vigorously to stroking, each stroke, though painless, producing a conspicuous local vasodilatation and whealing. It has previously been shown that these skins are unusual only in the degree in which they react, for similar reactions may be obtained from any skin by suitably grading or repeating the stimulus.

Believing, on the basis of previous observations, that the wheal is due to the release of small quantities of the substance named, we thought it might be possible by repeated stroking to release a quantity sufficient to yield a recognisable general response of the circulation. A comparatively large area of the trunk is stroked by means of a coarsely and bluntly toothed comb, the desire being to obtain a large number (100 or more) of closely set wheals almost simultaneously. If this is done upon a subject that has remained at absolute rest in a warm room for a sufficient control period, it is found that a slight but distinct general vasodilatation actually occurs. This is most readily detected in the face, which flushes perceptibly and displays a rise of temperature of about  $0.6^{\circ}\text{C}$  ( $0.3-1.1^{\circ}\text{C}$ ). The flush is not so visible in the arm and hand, though here also the temperature rises by almost equivalent amounts. A fall

<sup>1</sup> Work undertaken on behalf of the Medical Research Council.

of blood-pressure may be discerned, but usually the reaction is insufficient to cause this change. The rise of temperature comes to its height in about 7 minutes, or shortly after the local skin reaction is at its height, and lasts for a period of about 5-10 minutes more, by which time the skin reaction to injury has almost subsided. If the observation is carried out in exactly the same manner upon subjects in whom the skin shows no exaggeration of the usual response to stroking, the general reaction described does not occur. But these subjects, and those whose skins wheal, manifest equally a general reaction, of precisely the same type and lasting for the same period of time, when histamine (in doses of about 0.3 c.c. of a 1 in 3000 solution of the base) is introduced subcutaneously. We find in these observations the confirmation sought, namely, that the wheal is due to a histamine-like substance released in the skin by the stroke, and we can add that the quantity of substance released, in the case of the extensive whealing described, amounts, in terms of histamine-base, to about one-tenth of a milligramme. Further details of these observations will be published at a later date.

#### **Adrenaline vaso-dilation** By A. D. MACDONALD and W. SCHLAPP

Small doses of adrenaline (0.002 mg.) may depress the blood-pressure of the anaesthetised cat, and various explanations have been given for this phenomenon. It does not appear to have been realised or appreciated that this fall of pressure is very specially related to the anaesthesia, it is marked under ether and urethane, less marked under chloralose if no ether has been given just before. In the high pressure decerebrate cat from which all traces of ether have been thoroughly ventilated and after the circulation has had time to recover from the effects of anaesthetic, no such depressor response can be evoked, although it was well-marked shortly after decerebration and the level of blood-pressure remains scarcely changed. Depressor doses may now produce prolonged and considerable rises, often interrupted by a notch suggesting a double seat of action of the drug. On ether being supplied to such an animal the depressor response quickly returns.

If, as Burn and Dale<sup>(1)</sup> suggest, adrenaline vaso-dilation be due to the production in the lungs of minute amounts of a histamine-like substance, that substance can only be produced or liberated under the influence of the anaesthetic, for the blood-pressure response to small doses of histamine itself is little affected by the nature or degree of the anaesthesia in an animal with high blood-pressure.

**A substitute for blood fibrin in work on digestion**

By WINIFRED MARY CLIFFORD, King's College for Women, Kensington

Blood fibrin, in this country, is often difficult to obtain, needs long washing, and its strings are thick and of very varying diameter. A substitute was found in cod muscle fibrin which is readily obtainable, possesses fine, easily separable fibres, and is colourless.

The cod is freed from skin and bone and minced. The mince is covered with cold water and left 1—2 hours to remove soluble albumen. It is shaken at intervals. The water is strained off, and the mince boiled in a flask with more water for about one hour. It is re-strained and boiled again for another hour with fresh water. Finally the cod fibrin is wrung through a cloth.

If possible, it is advisable to shake the product with water on a shaker, in order to obtain a homogeneous mixture of the fibres from various parts of the fish muscle, but it is not absolutely necessary.

All the fibres are found separate and they can be kept under a 50 % mixture of glycerol and water in a similar way to blood fibrin.

Owing to the colourless nature of the protein, it is very suitable for the preparation of Congo Red and Carmine fibrins.

This preparation is particularly suitable for class purposes owing to the rapidity of digestion.

Actual colorimetric findings with 0.5 gram of blood and fish fibrins in each case using the blood fibrin as a standard are —

Time	Blood	Fish
$\frac{1}{2}$ hour	20 mm	15 mm
1 hour	20 mm.	10 mm
$3\frac{1}{2}$ hours	20 mm	20 mm

**Peculiar substance in the central nervous system of cats kept on autoclaved meat** By C. DA FANO (*Received Oct 4th, 1926*)

Since 1922-23 in the central nervous system of cats kept on autoclaved meat I noticed the occurrence of a substance which stained in various shades of purple-red with toluidine blue, thionine, mixtures of these two dyes and Giemsa's fluid, provided the material was fixed in formalin or formol-Müller or, very seldom, in alcohol and embedded in paraffin. In sections from celloidin blocks no trace of it could be seen. The substance in question was found chiefly in the white matter and was situated in the thickness of the tissue, viz. it was not a precipitate of the staining solutions used. It appeared in the shape either of small patches with irregular edges or of flower-like tops with a pseudo-

crystalline aspect. The dimensions of these peculiar formations varied extremely, some being as small as secretory granules, others measuring more than  $30\mu$  in diameter. They could be readily dissolved by placing the sections for a few hours in mixtures of absolute alcohol and ether, which explains their absence in material embedded in celloidin. They soon lost their bright purple colour after exposure of the specimens to sunlight. Subsequently the same substance was observed in sections from the spinal cord of apparently normal cats poisoned with chloroform or A.E.C. after an intravenous injection of chloralose.

In January, 1924, being in Florence, I went to see Buscaino with whose work I was acquainted. He kindly showed me a number of specimens and I formed the opinion that his "aciniform areas of disintegration" were similar to, but not identical with, the formations present in my specimens. On my return to London I was on the point of publishing my observations but I refrained owing to the doubt that such appearances might have been a mere artifact due, in part at least, to the way in which the material had been fixed. Particularly in the case of animals affected with deficiency diseases this seems more liable than usual to become altered under the influence of ordinary methods of histological investigation.

In June of this year Dr F. A. Pickworth brought to my laboratory some specimens showing the occurrence of similar phenomena. He had obtained them under experimental conditions which he proposed describing in a forthcoming note. Whereupon I suggested that he should add the content of this communication to his paper, but he desired that the results of our work should be published separately.

A definite opinion as to the nature of the appearances in question cannot be expressed at present.

**The effect of high and low oxygen pressure on the respiratory exchange during exercise** By A. E. CLARK-KENNEDY and TREVOR OWEN (*London Hospital Medical Unit*)

In a previous communication (1) we questioned the conception of the limitation of effort as being due to failure of the heart to maintain the  $O_2$  supply to the muscles, and pointed out that the symptoms of exhaustion are not comparable with those of heart failure.

*Experimental.* The respiration has been investigated during work of the same duration, but increasing  $O_2$  requirement. With increasing rate of work the  $RQ$ , pulmonary ventilation, percentage of  $O_2$  in the expired

air, and percentage of  $O_2$  requirement incurred as debt, rise, the percentage of  $O_2$  taken out of the inspired air and the percentage of  $CO_2$  in the expired air fall. Progressively more ventilation becomes necessary to effect the same  $O_2$  intake and  $CO_2$  output. Now Barr and Himwich(2) have shown that after exercise the  $CO_2$  carrying power of the blood is reduced and its  $C_H$  raised, due to lactate accumulation, observations indirectly supported by the work of A. V. Hill(3). Though primary overbreathing due to anoxæmia may play a part in their production, the changes described must be largely due to acidosis. The rise of  $C_H$  stimulates the respiratory centre and an excess of  $CO_2$  is blown off with rise of  $RQ$ . The reduced  $CO_2$  carrying power of the blood demands more ventilation to eliminate unit quantity and the alveolar  $CO_2$  tension falls, less  $O_2$  is taken out of the inspired air and the alveolar  $O_2$  tension rises. The ability to breathe is therefore important, not only in maintaining the  $O_2$  saturation of the blood as the circulation rate rises, but also in effecting the elimination of  $CO_2$  as the  $CO_2$  carrying power of the blood falls.

The respiration has also been compared during work of the same duration and  $O_2$  requirement, breathing 26 p.c., 21 p.c. (air), and 16 p.c.  $O_2$ . When 26 p.c.  $O_2$  is breathed, the  $O_2$  income is greater and the percentage of the  $O_2$  requirement incurred as debt less, the pulmonary ventilation and  $RQ$  are lower and the percentage  $O_2$  absorption and  $CO_2$  excretion higher, suggesting that the accumulation of lactate is less. When 16 p.c.  $O_2$  is breathed the findings are the opposite, suggesting greater lactate accumulation. Running all out breathing a high  $O_2$  mixture, the  $O_2$  income and debt are both greater (more work possible), and when breathing a low  $O_2$  mixture both less (less work possible), than when air is breathed.

*Theoretical.* Rise of  $C_H$  is probably the important physical basis of the psychical distress which limits voluntary effort. When the rate of work is such that the  $O_2$  supply to the muscles fails, so that lactic acid accumulates,  $CO_2$  elimination becomes progressively more difficult because (1) the  $CO_2$  carrying power of the blood is reduced, (2) progressively more ventilation is required to eliminate unit quantity, and (3) excess of  $CO_2$  has to be eliminated to keep down the  $C_H$  of the blood. The  $C_H$  of the blood therefore rises to the point at which effort ceases, not only on account of accumulation of lactate as the mechanism for  $O_2$  intake fails, but also on account of the accumulation of  $CO_2$  as the mechanism for the elimination of  $CO_2$  fails. Moreover, our high and low  $O_2$  experiments suggest an equilibrium between these two mechanisms, accumu-

lation of lactate when the  $O_2$  supply fails reduces the  $CO_2$  carrying power of the blood and demands more pulmonary ventilation to eliminate unit quantity, but the consequent increase in alveolar  $O_2$  tension increases the  $O_2$  saturation of the blood, delays the accumulation of lactate and raises the  $CO_2$  carrying power so that less ventilation is required to eliminate unit quantity. Raising the  $O_2$  pressure in the inspired air retards the accumulation of lactate and delays the retention of  $CO_2$ , for the same limiting rise of  $C_H$ , therefore, a greater accumulation of lactate is possible and a larger  $O_2$  debt can be incurred. Conversely, lowering the  $O_2$  pressure in the inspired air accelerates the retention of  $CO_2$ , and for the same limiting rise of  $C_H$ , the accumulation of lactate is less, and the  $O_2$  debt smaller.

*Conclusion.* Voluntary effort is limited by failure of the functional capacity of the cardio-respiratory system as a whole, not by premature failure of any one member of this team, *e.g.* the heart, or of one particular function, *e.g.*  $O_2$  intake rather than  $CO_2$  output, in extreme muscular exertion integration of function is maintained. When, however, the functional capacity of the heart is reduced by disease, on attempted effort integration of function can be maintained only by corresponding reduction in the functional capacity of breathing. Thus in compensated heart disease, the vital capacity of the lungs (themselves not diseased) is reduced (Peabody(4)). When, however, the limits of this protective reaction are reached, disintegration of cardio-respiratory function supervenes and blood accumulates in the veins with symptoms of congestive cardiac failure. From the standpoint of physiology, disease is to be regarded as disintegration of function. As disease progresses in one organ, integration is for a time maintained by readaptation of the functions of others, but sooner or later disintegration begins.

(1) Clark Kennedy, Bradbrooke and Owen. *Proc Physiol Soc* Dec. 12th, 1925 *Journ. Physiol* 61

(2) Barr and Himwich. *Journ Bio Chem* 55 pp 495, 525 1923

(3) Hill, Long and Lupton. *Proc Royal Soc B* 96 p 438 1924.

(4) Peabody and Wentworth. *Arch. Int. Med.* 20 p 443 1917

### **The effects of variations in $pH$ on the volume of red cells**

By A C HAMPSON and M MAIZELS (*Preliminary communication*)

Investigations were undertaken to show the effects of variations of  $pH$  on the volume of red cells. Mixtures of solutions of  $K_2HPO_4$ ,  $KH_2PO_4$ ,  $KOH$ , and  $H_3PO_4$ , of the same osmotic pressure, as determined cryoscopically, were used.

Hæmatokrit determinations of hirudinised blood were made in tubes drawn out into a sealed capillary. The tubes were centrifuged till the volume of red cells was constant and the mass translucent. The tubes were filled with solutions of potassium phosphate of varying  $pH$  but the same osmotic pressure. The red cells were mixed with the fluid, and after 30 minutes again centrifuged to constant volume. It was found that the volume of red cells in a solution of given concentration was minimal at  $pH$  8.1, and that in a solution with  $\Delta = 0.423$  (equivalent of 0.72 NaCl) the original volume of red cells taken from plasma was unaltered. This solution was therefore regarded as isotonic, and for further determinations, 1 c.c. of blood was suspended in 100 c.c. of this solution at  $17-19^{\circ}C$ , allowed to stand 30 minutes, and quantities of 5 c.c. centrifuged till the red cells were of constant volume. The supernatant fluid was replaced by solutions of varying  $pH$  but of the same osmotic pressure, in which the cells were suspended for 30 minutes and again centrifuged to constant volume. The  $pH$  of the supernatant fluid was taken and the volume of cells calculated as a percentage of the original volume.

It was found

(1) That in a solution of potassium phosphate ( $\Delta = 0.423$ ) the minimum volume of cells was equal to the minimum volume of cells in NaCl solution ( $\Delta = 0.528$ ), but in potassium phosphate the minimum was attained at  $pH$  8.1, whereas in NaCl this was reached at a point more acid.

(2) In potassium phosphate ( $\Delta = 0.423$ ) the minimum volume was found at  $pH$  8.1. In solutions more acid, the volume increased till a  $pH$  of 5.4, after which it fell sharply to another minimum at  $pH$  3 (approx.).

(3) On the alkaline side a gradual rise occurred as the  $pH$  increased till a  $pH$  of about 10.6, when the rise in volume became much more marked, hæmolysis occurring at a  $pH$  of about 11.0.

(4) At a given  $pH$  the volume increased with dilution. This was most marked at  $pH$  5.4 (approx.), less marked in solutions more acid than this, and at a  $pH$  of about 3.0, the percentage volume was the same, *irrespective of dilution*.

(5) In solutions more acid than 5.4, slight agglutination could be noted, and this became more marked till at  $pH$  3, agglutination was complete. In still more acid solutions, hæmolysis occurred, with the obvious presence of acid hæmatin.

(6) In solutions with  $\Delta = 0.320$  (equiv. of 0.54 p.c. NaCl), no hæmolysis occurred between  $pH$  3 and 10.8, but in solutions with





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**Herpes zoster and antidromic impulses<sup>1</sup>** By T. LEWIS and  
H. M. MARVIN (*Preliminary communication.*)

A series of researches upon the responses of the skin to a variety of injuries has led to the generalization that, whenever a lesion appears upon the skin, and this lesion consists essentially of reddening followed by whealing or blistering, the corresponding reaction of the vessels is due to the local release of substances having a histamine-like action. If this generalization is to hold good universally, it must include the phenomenon of herpes zoster, which is well known to be associated commonly with inflammatory lesions of the posterior root ganglion. Evidence accumulates to show that herpetic lesions may result from a variety of lesions of this ganglion or of the sensory nerve path upon which it stands, consequently, we have been brought to reinvestigate the nature of antidromic nerve action and to look for evidence that such action is due to the release of vasodilator substances in the skin. We stimulate peripherally a branch of the median nerve of cats from which the stellate ganglion of the same side has been removed three weeks previously, and we obtain flushing of the corresponding pads of the foot, as did Langley (1). A 20-second faradic stimulation produces a flush that lasts 5 to 10 minutes. We call attention to the long duration of the reaction, which in our view is itself most suggestive, since it exceeds that produced by stimulating the sympathetic vasomotor nerves. If, immediately before stimulating, the circulation to the limb is brought to a standstill and is so maintained for a period of time equivalent to that previously determined for the subsidence of the flush, and if the circulation is now restored to the limb, the pads all flush intensely. When this reactive hyperæmia fades, as it does within a minute or so, the pads supplied by the stimulated nerve still remain brightly coloured. The flush displayed by these pads subsides very gradually, its duration from the release of the circulation is equal to its duration, measured

<sup>1</sup> Undertaken on behalf of the Medical Research Council.

from the end of stimulation, when the circulation is undisturbed. That is to say, the vasodilatation produced antidromically is prolonged in our experiment by the period of circulatory arrest, or, stated otherwise, the subsidence of the flush is governed by the subsequent flow of blood to the skin. We believe that this can be interpreted only to mean that the flush is due to vasodilator substances released in the skin, that these substances are held in place during circulatory arrest, and are subsequently removed in the ordinary manner when the circulation is restored. To control these observations, we have investigated the vasoconstrictor effect upon the rabbit's ear of stimulating the cervical sympathetic, and the vasodilator effect produced in the glans penis of the cat by stimulating the pelvic nerve. In neither of these instances is the reaction held up by circulatory arrest.

We conclude that antidromic vasodilatation is due to the conveyance of impulses along the sensory nerves, not to the vessels, but to the tissues of the skin, and that these impulses release histamine-like substances in the skin (a trophic action), these substances then act on the minute vessels and produce vasodilatation in the territory of release. We conclude that herpes zoster is due to a similar release, produced by irritation of the sensory nerve tract. The conclusion of Doi (2), Krogh, Harrop, and Rehberg (3), and others, that capillaries possess a vasodilator innervation, is rendered untenable by these observations, since, according to our observations, the capillaries are reacting directly to the substances released.

Feldberg (4) has recently demonstrated antidromic vasodilatation in the rabbit's ear. We find that histamine dilates the minute vessels of the rabbit's ear and conjunctival mucous membrane when applied directly to these.

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PROCEEDINGS  
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**A muscle twitch lasting for hours** By K FURUSAWA.

The longitudinal abdominal muscle of *Holothuria nigra*, put in diluted sea water (10—20 p.c.), can respond to a single induction shock, and it requires several hours to relax completely. The contraction has the following properties: (1) the time required to reach a maximum is the same order as that in undiluted sea water, (2) the rate of relaxation depends upon the force exerted on the muscle, (3) this contraction can be set up repeatedly without any sign of fatigue, (4) the pull response during contraction is quite different from that which Hill and Gasser found with frog's muscle, (5) complete recovery of normal function is observed in sea water.

**The influence of one coloured light on the sensitivity of the eye to the same and other colours** By H E ROAF *From the Department of Physiology, the London Hospital Medical College (Preliminary communication.)*

If different regions of the spectrum excite different retinal mechanisms they should have comparatively little effect on each other. The degree to which one light interferes with another may be considered as a measure of the extent to which they act on similar mechanisms. In the results that follow the sensitivity is measured by the threshold to the coloured light.

Exposure of one part of the retina to light has a general effect in decreasing the sensitivity of other parts: this is the effect on the dark-light adaptation of the eye. All regions of the spectrum have a similar effect on the adaptation of the eye, namely that they decrease the sensitivity to blue and violet most, green next, and have very little effect on red.

When, however, the two lights stimulate the same area of the retina special differences are noted. Red light decreases the sensitivity to all regions of the spectrum. Green light decreases the sensitivity to green

and blue, but has little effect on red. Blue light has most effect on the sensitivity to blue, less on that to green and least on that to red.

These results differ from those of Burch(1), who says that the fatiguing light has an effect only on its own region of the spectrum. They also differ from the results of Troland(2), who states that "minutness due to one colour does not alter the luminosity of another colour to a degree differing appreciably from that in which it is altered itself." Allen(3) finds that exposure of the eye to coloured light affects several regions of the spectrum, but that there are certain transition colours at which no effect is produced.

A practical application of the results is that a blue screen is the best to protect the eyes from other lights, e.g. when motoring.

(1) Burch, G. J., *Phil. Trans.* 191 p. 1 1899

(2) Troland, L. T. *Abstract Bull. Nela Res. Lab. General Electric Co. Cleveland* p. 388 1922

(3) Allen, F. (Latest paper) *Journ. Optical Soc. Amer.* 13 p. 383 1926

### **Toxaemia from Liver Grafting** By D. T. BARRY and J. FREUD

Arising from an investigation of the rôle of the liver in the regeneration of blood after haemorrhage, in which one of us was engaged some time ago, we were led by the condition of dogs grafted with liver substance to test the effects of their blood on other animals.

Grafted intraperitoneally the animals generally showed symptoms of depression according to the weight of liver used in proportion to body weight. In those where three to five grams of liver from freshly killed dog were used per kgm. the results were all fatal after intervals of from 24 hours to three days (four experiments C, D, E, T). When the size of the graft was reduced to about 2.5 or 3 grams per kgm. it was again fatal in all cases, but two lasted five days (five experiments, F, G, H, J, M, J being a female with pup near term). When the graft amounted to about one gram per kgm., not exceeding 1.5 grams, a fatal result occurred in one (S) on the third day (five experiments K, L, S, U, V). U and V were killed for blood on the second day, and K and L were allowed to live for 15 days in apparently perfect health, when they were regrafted. No graft was actually weighed but similar pieces were

Table I comprises a few of the protocols showing the very toxic effects of the blood of grafted animals on others. The poison is elusive, occurs at a certain interval after operation and lasts a limited time. The earliest period in which it was found to be toxic was seven hours

after operation, and the latest twenty-eight and a half hours after grafting. We express no opinion as yet about the nature of this poison. Exposure and agitation of oxalated blood caused disappearance of toxicity in one sample, clotting caused disappearance in another, heating to 56° C in a third.

Death in the poisoned animals is very characteristic: respiratory spasm, cry, rigidity, stiffening of head and tail, post mortem twitchings are prominent signs. Positive effects from intravenous injection of this toxic blood were obtained in three cases without causing death, while the characteristic death described resulted in ten cases (nos 1, 2, 5, 6, 14, 15, 16, 17, 18, 19), a sufficient number to establish the main fact with certainty.

Table I.  
*Some Protocols of Experiments*

Series I. Toxicity for Others.					
Index	Date	Condition	Operation	Observations	General Remarks
D 5-5 K. ♀	15/11/26	Young, normal	30 gm. liver perit. cav	Recovered well	Soon became weak. Death 24 hrs. Toxicity 18 hrs.
Do	16/11/26	Very weak	Bled (full)	10 cc. fatal to No 1, 20 sec.	P M 50 cc choc fluid in peritoneum
G 6 K ♂	20/11/26	Healthy, young	15 gm. liver perit. cav	Well at once	Very well three days— then death on 4th day
Do.	21/11/26	Very good	5 cc blood from ear	Blood fatal to dog 6 5 K. (20 sec.)	Blood of dead dog non- toxic. Toxicity 20 hrs.
J 9 K. ♀	24/11/26	With pup. near term	12 gm. liver perit.	Easy rapid op	Took food very soon, had seven puppies next day
Do.	25/11/26	Good	10 cc. bl. ear	Inj no 5 (lact.)	No effect on no 5 (7 K.)
Do	Do	Do	5 cc bl. ear	Inj no 6 (ord.)	Death of no 6 in 40 sec.
L 5-5 K. ♂	24/11/26	Young, normal	7-8 gm. liver perit.	Rapid recovery	Took food etc. soon after op Toxic 20 hrs.
Do.	25/11/26	Good	5 cc. bl. ear	Inj no 5 (lact.)	Death of no 5, 40 sec.
V 4 1/2 K. ♀	6/12/26	Healthy young	6 gm. liver perit.	—	Quick recovery Toxic 7 hrs.
Do.	11.45 a.m.	—	10 cc. bl. ear	Inj no 14 (11 K.)	Resp effects only
Do.	Do 4 p.m.	—	10 cc. bl. ear	Inj no 14	Death 1 min., cry, rigid.

**Impulses in the optic nerve** By E. D. ADRIAN and  
RACHEL ECKHARD (*Preliminary communication*)

The electric responses developed in the retina on exposure to light have been studied repeatedly. They are complex changes, generally regarded as made up of three or more components, and though they give a valuable index of the activity of the retina they give no information

as to the form in which this activity is transmitted to the brain, i.e. as to the nature of the impulses in the optic nerve fibres. The short length of the optic nerve and the large number of fibres in it makes it less easy to record the electric responses than is the case with a peripheral sensory nerve, but we have succeeded in doing so in the optic nerve of the conger eel (*Conger vulgaris*). This nerve contains relatively few fibres (under 10,000) and may be as long as 2 cm. The isolated eye and optic nerve preparation is arranged with electrodes leading from two points on the nerve to a three or four valve amplifier and the capillary electrometer. An opal glass screen is illuminated by a lamp at varying distances, the area of the screen is controlled by an iris diaphragm and its image can be focussed on the retina by lenses. As a rule the nerve is free from action currents when the eye is in darkness, but on illumination a rapid series of oscillations appear in the record (Fig 1). The usual controls have

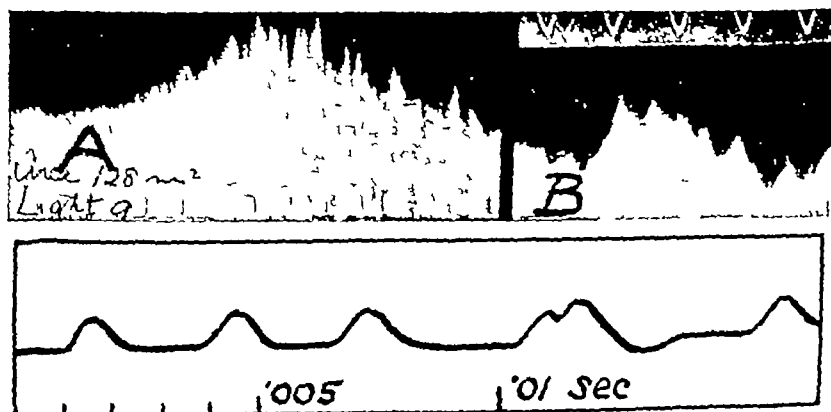


Fig 1 A Action currents in optic nerve on exposure to light. Film record. Time marker (white lines) gives intervals of  $\frac{1}{3}$  sec. Light turned on just before film begins.

B Ditto, plate record. Time marker (above) gives  $\frac{1}{3}$  sec intervals.

Fig 2 Analysis of another electrometer record showing time relations of action currents.  $T=16^{\circ}\text{C}$ .

been made to exclude artefacts. Analysis of records on plates moving at 80 cm per sec shows an irregular succession of mono- or diphasic action currents each lasting for about 0015 sec. The majority seem to conform to a standard size and duration though there may be considerable overlapping. The maximum frequency occurs at or near the beginning of the response and declines rapidly. There is usually a renewed outburst of impulses when the light is turned off (as there is in the retinal

response) The frequency on illumination varies with the area and the intensity of the light, though we are not yet in a position to give accurate values. Owing to the rapid decline in frequency with steady illumination a moving light or shadow produces a much greater effect than a stationary light.

Points of some interest have arisen in connection with the latent period, the frequency in each fibre, etc., but our records leave no doubt that the discharge in the optic nerve is of substantially the same type as that in other sensory nerves. This is illustrated in Fig 3, which

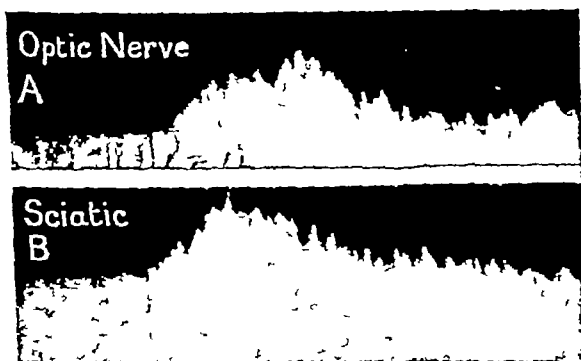


Fig 3 A. Action currents in optic nerve on exposure to light.  
B. Action currents in frog's sciatic produced by tension on gastrocnemius.

compares the response in the eel's optic nerve with that in the frog's sciatic when the gastrocnemius is stretched. Apart from the larger size of the action currents in the sciatic (which has fewer fibres) the records are almost indistinguishable.

### The ultra-violet absorption spectra of cerebro-spinal fluids

By F CAMPBELL SMITH. (*Preliminary note*)

Judd Lewis in 1922 obtained the quantitative absorption spectra of the serum proteins, and in 1923 Ward obtained those of some of the more important amino acids.

Other workers have since shown that the absorption bands of proteins are due to the amino acids contained within their molecule.

Cerebro-spinal fluid has the following advantages over the other body fluids in this type of work.

1. It can be obtained directly from the subject without contamination.
2. It is optically clear.

3 The concentrations of protein and other substances in it are within the range of the Hilger sector photometer and so it does not require dilution

4 It is free from pigment, both in the normal and majority of pathological conditions

It is, therefore, the one body fluid which can be investigated by this method without the risk of introducing error by manipulative technique

The normal absorption curve has been obtained and compared with those of various pathological conditions. Curves which appear to be typical of tuberculous meningitis and of tabes dorsalis respectively have been found

It is suggested that the method may be of diagnostic value

The curves and details of the technique will be published in a subsequent communication



PROCEEDINGS  
OF THE  
PHYSIOLOGICAL SOCIETY,  
*January 22, 1927*

**Blood pressure in the two- three-day chick embryo**

By LEONARD HILL and Y. AZUMA.

Fertile eggs placed on a canvas tray with a dish of water below were incubated for two and a half to three days in the hot room ( $39-40^{\circ}\text{C}$ ). One-third of the upper part of the shell was cut away with the egg held lengthwise in a bath of warm Ringer solution using great care so as not to touch the embryo or vascular area inside. The embryo with its S-shaped beating heart surrounded with the vascular area together formed an area of about 2 cm. diameter. Just outside the vascular area a cut was carried round with scissors and the thin membrane containing the embryo and vascular area thus set free without damage to any of the blood vessels. This membrane was floated on to a histological section lifter and gently transferred to a small sheet of "cellophane" (a thin transparent acetyl cellulose membrane) which was placed in position over the instrument (designed by Roy and Graham Brown) for measuring capillary pressure. This instrument consisted of a small metal chamber floored with glass and roofed with a loosely stretched sheet of peritoneal membrane. A T-tube connected the chamber on the one hand with a water manometer, on the other with a tube by which the pressure could be raised. By moving the sheet of cellophane which carried the embryo (and its vascular area) this was brought into the right position, and a glass plate was then brought down so as to cover one vitelline artery and vein and part of the vascular area, leaving the heart and much of the embryo and vascular area uncovered. When the pressure was raised in the chamber of the instrument the peritoneal membrane swelled and pressed the covered part of the vascular area against the glass plate. The blood vessels were thus compressed between the swollen peritoneal membrane and the glass plate and the pressures required to alter or stop the flow were easily read on the manometer. Everything had to be arranged right at the first adjustment because the vessels of the embryo are so tender that they tear if any attempt is made to alter the position of the glass plate. The chamber of the instrument was closely surrounded with another chamber with flat top on which the outer part of the sheet of cellophane rested, through this outer chamber water circulated at  $40^{\circ}\text{C}$ . An irrigation tube was also arranged so as to run

a stream of Ringer's solution at 40°C on to the embryo. By these means the embryo and the vascular area were kept normal and the heart beat and circulation proceeded with unabated vigour. Using a low-powered microscope the red corpuscles could be seen travelling with pulsatile movement through the arteries and continuously flowing through the capillaries and veins. The flow was rapid enough to make the movement of the corpuscles only visible at the border of the lumen of an artery.

We found that a pressure of 1.5–2 cm. of water sufficed to stop and reverse the flow in the vitelline arteries while 0.5 cm. pressure of water quickly applied sufficed to check the blood stream in the capillaries. These measurements confirm those previously made by L. Hill, and his conclusion that in tissues generally a very small difference of pressure maintains the capillary blood flow. They show that a very small pressure (2 cm. H<sub>2</sub>O) suffices to maintain a flow throughout the embryonic area, and that the range of pressure in the vascular system of the two and a half day old chick is from 2 cm. of water to zero. This vascular system is comparable in the adult to a vascular area consisting of terminal arterioles branching into capillaries, the capillaries uniting again to form terminal venules. It is evident that in the chick embryo interchange between blood and tissue cells goes on with a very low capillary pressure, and that such a low pressure suffices for the carrying out of the processes of life.

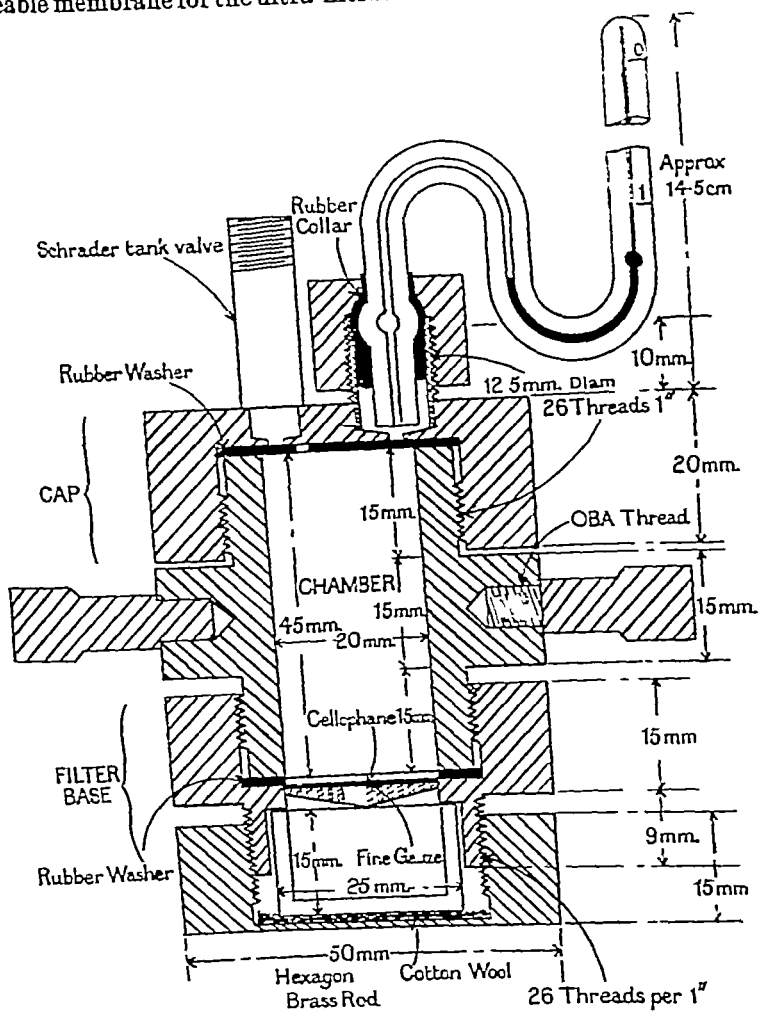
**The relation of alkali to the parasympathetic** By R. J. S. McDOWALL, King's College, London (*Preliminary communication*)

The injection of alkali intravenously into cats under chloralose and undisturbed results in a marked slowing of the heart, constriction of the pupil and increased gastric tone. This relation of alkali to the size of the pupil and to gastric tone has already been observed by many independent workers.

In the case of the heart the slowing is not to be considered a grossly toxic action, for the state of the animal is such that it responds with great readiness to all forms of sensory and sympathetic stimulation by an increased heart-rate while at the same time the pupil dilates. In the early stages the vagus restraint of the heart is very marked, but later section of the vagi or the administration of atropine do not increase the heart-rate. The effect of stimulation of the peripheral end of the vagus is much increased. These results taken together with the fact that CO<sub>2</sub> has the opposite effect suggest that the so-called sympathetic-parasympathetic balance is closely related to acid-base equilibrium.

**An easily constructed ultra-filter** By R S AITKEN  
and H D KAY *Medical Unit, London Hospital*

Commercial cellophane has been found to serve very well as a semi-permeable membrane for the ultra-filtration of undiluted serum or plasma



The apparatus has been designed for the ultra-filtration of small quantities (3 to 5 c c) through cellophane<sup>1</sup> at pressures up to six atmospheres, which are conveniently produced by a motor tyre pump It will give

<sup>1</sup> Thickness No 253—obtainable from the British Cellophane Company 7, Bird Street W 1

5 c c or more of protein-free ultra-filtrate in two hours Its general features will be obvious It is made of brass, and the parts, of the dimensions shown, can be conveniently turned from a 2 in hexagon brass rod, they are subsequently nickel-plated It consists of

A. *A cap*, carrying a Schrader "tank" valve<sup>1</sup> which is soldered in The manometer is made by sealing together two capillary tubes of different bore, the smaller above, the larger below Mercury is introduced, and the upper end sealed The portion of the narrow bore tube containing air is graduated by measurement to give an approximate indication of the pressure in atmospheres A large excursion of the meniscus in the narrow graduated part is accompanied by a much smaller excursion of the meniscus in the wide part The lower end of the manometer tube has a small symmetrical bulb blown on it, this is surrounded by a collar of rubber tubing, and is held in position in its socket by a perforated brass nut made with a shoulder and screwed tightly on to it, as shown in the diagram

B *A cylindrical chamber*, on to which the cap is screwed, with the intervention of a disc-shaped rubber washer, perforated by a small hole, this prevents liquid from reaching the valve or the manometer by splashing Two large brass screws in the wall of the chamber enable it to be firmly gripped in assembling the apparatus

C *A filter-base*, in the form of another screw cap, with many fine perforations Above the perforated part is placed a piece of fine silver gauze (60 meshes to the inch), then a disc of cellophane, then an annular rubber washer, and the whole screwed tightly into place There is slight crinkling, but no tearing of the cellophane, the crinkling may be minimised by a trace of vaseline on the upper surface of the rubber washer

D *A receiver*, which is a shallow glass dish, placed on a pad of moistened cotton wool, and held in position by an additional screw cap, having no washer

If a filter with greater capacity or greater filtering area is desired, the measurements may be altered accordingly

For the brass-work of the original apparatus, and for the accompanying drawing, we are indebted to our mechanic, Mr J McCarthy The filter may now be obtained from Herbert E Kendrick, 342, St John Street, E C 1

<sup>1</sup> Obtainable from A. Schrader's Son, Inc, Tyre Valve Manufacturers, 29, New Street  
S W 1

**A simple colorimeter lamp** By H D KAY  
*Medical Unit, London Hospital*

For accurate colorimetric work it is essential to have a steady, and therefore, at least in this country, an artificial source of light. It is also necessary to cut down to a minimum any adventitious light falling upon either the eye or the colorimeter.

Stanford (1), for use with his dilution colorimeter, described a colorimeter lamp in which the light from a Sheringham daylight lamp was reflected from a white paper surface through a hole in the side of a wooden box, to give a source of illumination of unvarying quality. The lamp described was of somewhat inconvenient size and shape for employment with the ordinary laboratory colorimeter, and the cost of the Sheringham daylight lamp made the adoption of this device expensive.

The same principle of the reflection of light from a white paper surface has been utilised and new useful points incorporated in the making of a small, inexpensive and convenient colorimeter lamp, which may be used, in place of the costly lamp houses which are on the market, with any of the common types of colorimeter (Dubosq, Kober, Bausch and Lomb).

The source of light is two Osram 30 watt "striplite" lamps "without reflectors," each  $8\frac{1}{2}$  in overall length. (For exact position of lamps see figs.) The box holding the two lamps is of  $\frac{1}{2}$  in white-wood,  $11\frac{1}{2} \times 7\frac{1}{4} \times 5\frac{1}{2}$  in outside dimensions, each of the two  $11\frac{1}{2} \times 7\frac{1}{4}$  in faces being perforated by a  $5\frac{1}{2} \times 2\frac{1}{2}$  in opening, the bottom of the opening being  $\frac{3}{4}$  in from the bottom of the box. A piece of tinned sheet-iron, on which thick white typewriting paper has been evenly pasted, reflects the light through a sheet of Chance's rough surface daylight glass<sup>1</sup>,  $6 \times 3$  in, on to the mirror of the colorimeter. The other opening in the box is closed by a sliding panel of the same size as the daylight glass, with which it is interchangeable. When very strong direct illumination is desired, the box is turned upside down and the panel removed, the daylight glass being inserted if required. A hinged door at one end of the box permits of the removal of the lamps, glass, panel and reflector, which are all detachable. It has been found that the most convenient way of construction is to make the box in two longitudinal halves and then screw these together.

A coat of dull white paint inside, and dull black outside, together with three screw points on each face of the box which is likely to touch

<sup>1</sup> Obtainable from Chance Bros., Smethwick, Birmingham

the bench, to give the whole the stability of a three point suspension, complete the lamp itself (see Figs 1 and 2) It is advisable, in order that the colorimeter once adjusted to the light source may not be accidentally moved, to fit the colorimeter base with three similar, fairly sharp points

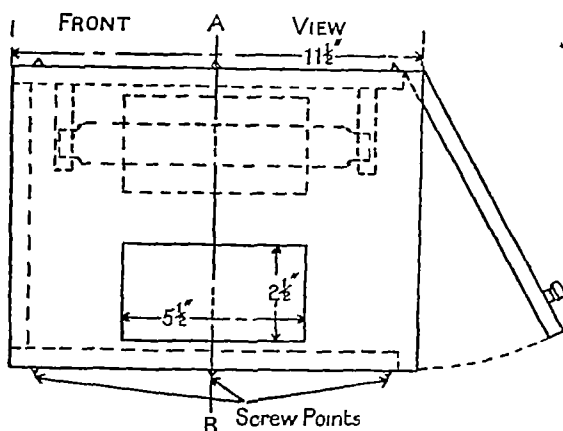


Fig 1

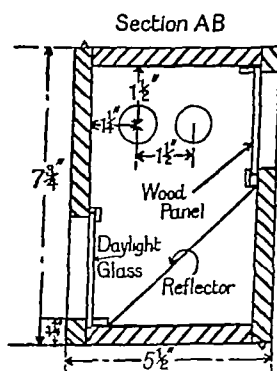


Fig 2

A screen of some kind, to cover lamp, colorimeter and the head of the observer, is a great advantage, and may be readily made of three-ply wood, painted dull black inside. The arrangement is shown in the figure

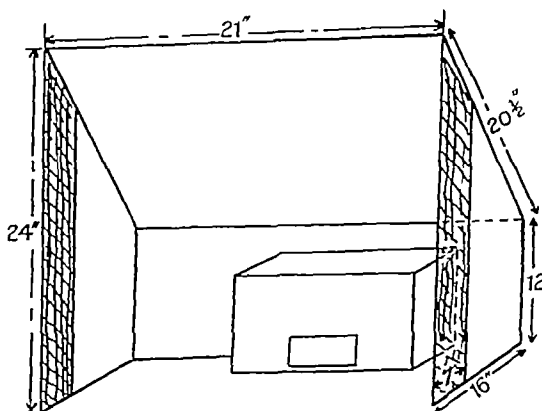


Fig 3

It is advisable, when using the Bausch and Lomb instrument, which is, in the experience of the present writer, by far the most satisfactory instrument for general purposes, to make the front 7 inches of the side

of the screen of thick black cloth (shaded in Fig 3), so that the scale may be read without difficulty

The colorimeter lamp is obtainable from Messrs Baird and Tatlock, Cross Street, Hatton Garden, E C 1

## REFERENCE.

- 1 Stanford Biochem. Journ. 17 p 839 1923

**The effect of alkali on blood sugar** By M A BATTIE  
and R J S McDOWALL, King's College, London

The injection of 2 c c saturated sodium bicarbonate solution intravenously into a cat under amytal anæsthesia causes a definite but temporary fall of blood sugar which may subsequently be succeeded by a rise. A similar fall has been obtained in man when 20 grams have been taken by the mouth. Confirmatory evidence exists in the literature

In view of the work of Clark and of Macleod and his co-workers the results support the suggestion that the action of alkali is related to parasympathetic activity, both possibly acting upon the same structures